abbexa®

Deoxynivalenol (DON) ELISA Kit

Catalog No.: abx364878

Size: 96T

Storage: Store at 2-8°C for up to 1 year. Once opened, store at 4°C and use within a month.

Application: For quantitative detection of Deoxynivalenol in feed and grain.

Sensitivity: 3 ppb (ng/ml)

Detection Limit: Grain, Feed – 150 ppb

Cross-reactivity: Deoxynivalenol ($C_{15}H_{20}O_6$) –– 100%, 3-Acetyldeoxynivalenol ($C_{17}H_{22}O_6$) – < 1%

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. The well plate is pre-coated with coupled antigen. The standards, samples and antibody solution are added to the wells and incubated. After washing away the unbound conjugates, HRP-conjugate is added to each microplate well. After TMB substrate solution is added, only wells that contain Deoxynivalenol 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 Deoxynivalenol amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Deoxynivalenol can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standards - 0 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb, 243 ppb, 1 ml each
3. HRP Conjugate reagent: 11 ml
4. Antibody Solution: 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. Reconstitution Buffer (2X): 50 ml
10. Plate sealer: 3 pieces
11. Hermetic bag: 1

Materials Required But Not Provided

1. 25°C incubator and precision balance
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Homogenizer
10. Centrifuge

Reagents Required But Not Provided

1. Deionized water

Protocol

A. Preparation of sample and reagents

1. Preparation of Sample pretreatment solutions

- **Solution 1 – Reconstitution Buffer**
  Dilute the 2X Reconstitution Buffer 2-fold with deionized water - i.e. add 50 ml of 2X Reconstitution Buffer to 50 ml of deionized water to make the 1X Reconstitution Buffer solution.
- **Solution 2 – Wash Buffer**
  Dilute the 20X Wash Buffer 20-fold with deionized water - i.e. add 40 ml of 20X Wash Buffer to 760 ml deionized water to make the 1X Wash Buffer solution.

2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Deoxynivalenol is unevenly distributed in samples, therefore it is recommended to assay multiple samples.

- **Grain *(rice, corn, millet)* and Feed:** Homogenise the sample. Weigh 2 g of homogenised sample and add to a 50 ml centrifuge tube. Add 10 ml of deionized water; oscillate for 5 mins, and then centrifuge at 4000 rpm for 10 mins at room temperature. Collect 0.1 ml of supernatant and transfer to a new tube. Add 0.9 ml of 1X Reconstitution Buffer, and thoroughly mix. Aliquot 50 µl and assay immediately.
  Note: Sample dilution factor: 50, minimum detection dose: 150 ppb.
- **Corn Skin, Wheat Bran, and other Strong Water Absorption Feed:** Homogenise the sample. Weigh 2 g of homogenised sample and add to a 50 ml centrifuge tube. Add 10 ml of deionized water; oscillate for 5 mins, and then centrifuge at 4000 rpm for 10 mins at room temperature. Collect 0.1 ml of supernatant and transfer to a new tube. Add 0.9 ml of 1X Reconstitution Buffer, and thoroughly mix. Aliquot 50 µl and assay immediately.
  Note: Sample dilution factor: 100, minimum detection dose: 300 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.

» Please bring sample slowly to room temperature.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

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 or triplicate. Add the solution at the bottom of each well without touching the side walls. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles.
2. Add 50 µl of the standard solutions into the standard wells.
3. Add 50 µl of prepared sample into test sample wells.
4. Immediately add 50 µl of Antibody solution to each well, and cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 37°C for 30 mins.
5. 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 minute 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.
6. Add 100 µl of HRP Conjugate Reagent into each well.
7. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 37°C for 30 mins.
8. Repeat the wash step as described in step 5.
9. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B into each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate in the dark at 37°C for 15 mins. Please note that the 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. 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 (reference wavelength 630 nm) immediately.

This assay is competitive, therefore there is an inverse correlation between Deoxynivalenol concentration in the sample and the absorbance measured.

Absorbance (%) = $(A / A_0) \times 100$
A: Mean absorbance of Standards (3 ppb - 243 ppb) or Samples
A_0: Mean absorbance of 0 ppb Standard

Create a graph with the log of the standard concentration (y-axis) against the Absorbance (%) (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.

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. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. 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 dump the residual solution back into the vial.