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Product Information

Deoxynivalenol ELISA Kit
for grains, cereals, and animal feed

Catalog Number **SE120009**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Deoxynivalenol (DON, 3α,7α,15-Trihydroxy-12,13-epoxytrichothec-9-en-8-one) is a water-soluble metabolite of trichothecene mycotoxins that are produced by *Fusarium* fungi, in particular *F. graminearum*.[1] *Fusarium* fungi are known to infect barley, wheat, and corn (maize). Known to inhibit protein synthesis,[2] DON is toxic and causes immunological disturbances.[3] In particular, DON is known to induce feed refusal and vomiting in pigs.[2] This property gives DON the colloquial name of vomitoxin.[4]

This Deoxynivalenol ELISA Kit is a solid-phase direct competitive enzyme immunoassay. A deoxynivalenol-specific antibody is coated to a polystyrene microwell. Toxins are extracted from a ground sample with distilled or deionized water. The extracted sample and HRP-conjugated DON are mixed and added to the antibody-coated microwell. DON from the extracted sample and HRP-conjugated DON compete to bind with the antibody coated to the microwell. Microwell contents are decanted, and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added, which develops a blue color in the presence of HRP. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of DON in the sample or standard. Therefore, as the concentration of DON in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added, which changes the chromagen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD_{450}). The optical densities of the samples are compared to the optical densities of the kit standards, and an interpretative result is determined.

The Deoxynivalenol Assay is a competitive ELISA intended for the quantitative detection of DON in cereal grains and other commodities including animal feeds.

Components

1. Deoxynivalenol Microplate (941DON01M): 96 wells (12 × 8 well strips) in a microwell holder coated with a mouse anti-deoxynivalenol antibody
2. Deoxynivalenol Standard (943S1DON01M, Black Cap): 6 vials, 1.5 mL/vial of deoxynivalenol at 0, 10, 20, 50, 100, and 200 ng/mL
3. Deoxynivalenol HRP-Conjugate (944MDON01, Green Cap): 12 mL of deoxynivalenol conjugated to peroxidase in buffer with preservative
4. TMB Substrate (916T001, Blue Cap): 12 mL of stabilized 3,3′,5,5′-tetramethylbenzidine (TMB)
5. Stop Solution (946P001, Red Cap): 12 mL of Acidic Solution
6. PBST Wash Buffer Powder (915X001): 1 packet of PBS with 0.05% TWEEN® 20. Bring to 1 liter with distilled water and store refrigerated.
7. Mixing Wells (Green): 96 non-coated wells (12 × 8 well strips) in a microwell holder

Reagents and Equipment Required but Not Provided.

1. Grinder sufficient to render sample to particle size of fine instant coffee
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes to deliver 100–200 µL volumes
4. Collection Container: minimum 125 mL capacity
5. Graduated cylinder: 100 mL
6. Distilled or deionized water
7. Filter Paper: Whatman® #1 or equivalent
8. Filter funnel
9. Absorbent paper towels
10. Graph paper or computer and software for ELISA data analysis

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with deoxynivalenol. Wear protective gloves and safety glasses when using this kit.

Storage/Stability
Store reagents at 2–8 °C, and do not use beyond expiration date(s). Never freeze the kit components. HRP-labeled conjugate and TMB Substrate are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

Procedures
Before doing the assay, prepare a waste container as a receptacle for kit waste. Eject contaminated pipette tips and all other related materials into this container. Following completion of the assay, treat the container with sufficient 5-6% sodium hypochlorite (NaOCl) to saturate the container's contents, about $1/10^{th}$ the volume of the container. 5-6% NaOCl will denature the mycotoxins and neutralize the waste, which renders the waste safe for disposal. Invert the container several times to coat all waste thoroughly.

(In case of an accidental toxin spill, treat the spill surface with 5-6% NaOCl for a minimum of 10 minutes, and then with 5% aqueous acetone. Wipe dry with absorbent paper towels.)

Extraction
Notes: Samples must be collected according to established sampling techniques.

The samples to be tested should have a pH of 7.0 ± 1.0, as excessive alkaline or acidic conditions may affect the test results.

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen).
2. Weigh out a 20 g ground portion of the sample. Add 100 mL of distilled or deionized water.
   Note: The ratio of sample to water is 1:5 (w/v).
3. Mix by shaking in a sealed container or in a blender for a minimum of 3 minutes.
4. Allow the particulate matter to settle. Filter 5–10 mL of the extract through Whatman #1 filter paper (or equivalent). Collect the filtrate.
5. Dilute the sample 10-fold by adding 1 mL of sample extract to 9 mL of PBST Wash Buffer.
6. The sample, at a final dilution of 50-fold, is now ready for testing. The standards require no pre-dilution before use.

Assay
Note: It is recommended that a multichannel pipettor be used for the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

Bring all reagents to room temperature (19–27 °C) before use.

1. Reconstitute the PBST buffer in distilled water. The remaining content of the packet may be washed out with a gentle stream of distilled water. Store the reconstituted buffer refrigerated, when not in use.
2. Place one Mixing Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody-Coated Microwells in another microwell holder.
3. Dispense 200 µL of the HRP-Conjugate into each Mixing Well.
4. Using a new pipette tip for each, add 100 µL of each Standard and Sample to appropriate Dilution Well containing HRP-Conjugate. Mix by priming the pipettor at least 3 times.
   Note: Operator must record the location of each Standard and Sample throughout the test.
5. Using a new pipette tip for each, transfer 100 µL of contents from each Dilution Well to a corresponding Antibody-Coated Microwell. Incubate at room temperature for 15 minutes.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBST Wash Buffer, then decanting the buffer into a discard basin. Repeat wash for a total of 5 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
8. Measure the required volume of Substrate Reagent (1 ml/strip or 120 µL/well) and place in a separate container. Add 100 µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
9. Measure the required volume of Stop Solution (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µl in the same sequence and at the same pace as the Substrate was added.
10. Read and record optical density (OD) of each microwell with a microplate reader using a 450 nm filter.
11. Setting the zero standard as 100% binding (Bo), calculate % binding (%B) for each standard and sample as a percentage of the zero binding (%B/Bo).

Results
Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0) standard against the DON content of the standard. Unknowns are measured by interpolation from the standard curve.

The information on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted 5-fold with distilled water, followed by a further 10-fold dilution in PBST Wash Buffer. Thus the level of deoxynivalenol shown by the standard must be multiplied by 50 in order to indicate the μg of deoxynivalenol per gram of commodity (ppm), as follows:

Standard ng/ml	Commodity (ppm)
0.0	0.0
10.0	0.5
20.0	1.0
50.0	2.5
100.0	5.0
200.0	10.0

If a sample contains DON at a concentration greater than the highest standard, it should be diluted appropriately in distilled or deionized water, and retested. The extra dilution step should be taken into consideration when expressing the result.

Recovery Sample Data
Recovery was determined by spiking 2.5 ppm of DON into the following commodities:

Commodity	% Recovery
Animal feed	73.9
Corn	82.1

Tests on certified reference material for wheat gave these recovery data:

Wheat Reference Sample (ppm)	% Recovery
0.5 ± 0.07	80.0
2.5 ± 0.1	85.6
5.1 ± 0.3	90.7

Reproducibility
Intra-Assay: CV <5%
Inter-Assay: CV <8%

Limit of Detection (LOD)
Limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of a DON-free commodity extract. As different commodities generate somewhat different zeros because of 'matrix inhibition' effects, it follows that the LOD is commodity-specific and should be measured empirically for each different commodity.

References

1. Audenaert, K. *et al.*, Deoxynivalenol: A Major Player in the Multifacted Response of Fusarium to its Environment. *Toxins (Basel)*, **6(1)**, 1-19 (2014).
2. Pinton, P., and Oswald, I.P., Effect of Deoxynivalenol and Other Type B Trichothecenes on the Intestine: A Review. *Toxins (Basel)*, **6(5)**, 1615-1643 (2014).
3. Sobrova, P. *et al.*, Deoxynivalenol and its toxicity. *Interdiscip. Toxicol.*, **3(3)**, 94-99 (2010).
4. Pierron, A. *et al.*, Impact of two mycotoxins deoxynivalenol and fumonisin on pig intestinal health. *Porcine Health Manag.*, **2**, 21 (2016).

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