Helica® Deoxynivalenol ELISA

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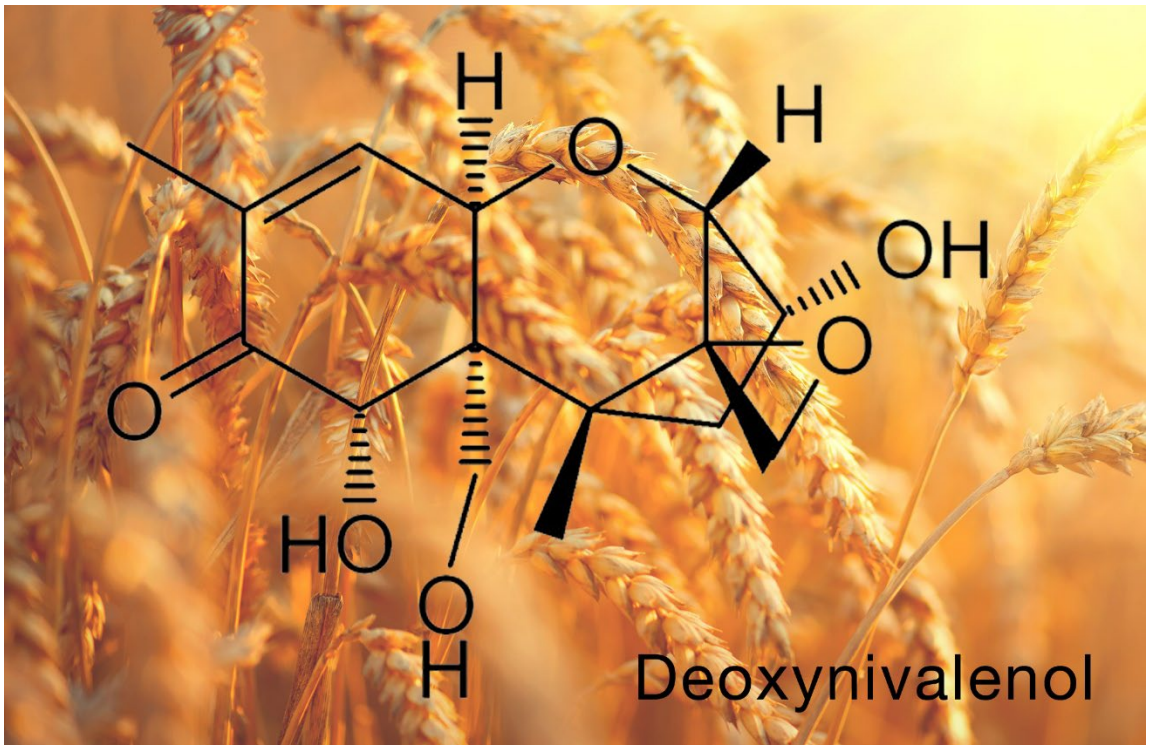

Helica® Deoxynivalenol ELISA

For the quantitative detection of deoxynivalenol in cereal grains and other commodities including animal feeds.

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Introduction – Deoxynivalenol (Vomitoxin)

Deoxynivalenol (DON) is a low molecular weight metabolite of the trichothecene mycotoxin group produced by fungi of the *Fusarium* genus, particularly *F. graminearum*. These fungi occur widely and will infect barley, wheat and corn (maize). Deoxynivalenol is highly toxic, producing a wide range of immunological disturbances and is particularly noted for inducing feed refusal and emesis in pigs, hence the alternative name vomitoxin.

Intended Use

Hygiena's Helica® Mycotoxin ELISA kits are user-friendly, cost-effective kits for the detection of mycotoxins in a wide range of commodities including animal feeds, grains, corn and animal urine, designed to protect humans and animals from dangerous side effects of mycotoxins.

The Helica Deoxynivalenol ELISA assay is a competitive enzyme-linked immunoassay intended for the quantitative detection of deoxynivalenol in cereal grains and other commodities including animal feeds. For research use only. Not for use in diagnostic procedures.

Data obtained from assays should not be used for human diagnostic or human treatment purposes. Assays are not approved by the United States Food and Drug Administration or any other US or non-US regulatory agency for use in human diagnostics or treatment. Helica assays should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program.

Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under the supervision of technically qualified persons.

Principle of the Method

The Helica Deoxynivalenol (DON) ELISA assay 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 DON bound to horseradish peroxidase (HRP) 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. After this incubation period, the contents of the wells are decanted, washed and a HRP substrate is added which develops a blue color in the presence of the enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of DON in the standard or sample. 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 chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD450). The optical densities of the samples are compared to the ODs of the kit standards and a result is determined by interpolation from the standard curve.

Kit Contents

Package/ Number	Component	Description
1X Pouch	Antibody-coated microwell plate	96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-deoxynivalenol antibody, *Ready-to-Use*.
1X Plate	Dilution plate	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use.* (Mixing wells)
6X Vials	Standards	1.5 mL/vial of Deoxynivalenol at the following concentrations: 0.0, 10.0, 20.0, 50.0, 100.0 and 200.0 ng/mL in deionized water, *Ready-to-Use*.
2X Bottles	Conjugate	2 x 12 mL of Deoxynivalenol conjugated to peroxidase in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	PBS-T powder	PBS with 0.05% Tween® 20, bring to 1 Liter with distilled water and store refrigerated. (Wash buffer)

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Materials Required but Not Provided

- Grinder sufficient to render sample to a particle size of fine instant coffee
- Collection tube: Minimum 125 mL capacity
- Balance: up to 20 g measuring capability
- Graduated cylinder: 100 mL
- Distilled or deionized water: 100 mL per sample
- Filter paper: Whatman #1 or equivalent
- Filter funnel
- Pipettor with tips: 100 µL and 200 µL
- Timer
- Wash bottle
- Absorbent paper towels
- Microplate reader with 450 nm filter

Storage and Shelf Life

- Store reagents at 2 to 8 °C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- HRP-labeled conjugate and TMB-substrates are photosensitive and are packaged in a light-protective opaque bottle. Store in the dark and return to storage after use.

Precautions and Waste Disposal

General Precautions:

- Bring all reagents to room temperature (19 to 25 °C) before use.
- Do not interchange reagents between kits of different lot numbers.
- Do not use solutions if cloudy or precipitate is present.
- Do not return unused reagents to their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.
- During the sample extraction, avoid cross-contamination.
- Devices, such as a blender, must be cleaned after each sample preparation.
- Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.

Safety Precautions:

Mycotoxins (aflatoxins, trichothecenes and others) are well-known human carcinogens and are considered highly toxic. Because mycotoxins can cause human illness, appropriate safety precautions must be taken and personal protective equipment worn when handling samples, reagents, glassware and other supplies and equipment that potentially could be contaminated with mycotoxins.

- Before using this assay, please review the Safety Data Sheet(s) available at www.hygiena.com.
- Refer to your site practices for safe handling of materials.
- It is strongly advised that protective gloves, a lab coat and safety glasses be worn at all times while handling mycotoxin kits and their respective components. Consider all materials, containers and devices that are exposed to samples or standards to be contaminated with mycotoxins.
- Never pipette reagents or samples by mouth.
- Standards are flammable. Caution should be taken in the use and storage of these reagents.
- The stop solution contains sulfuric acid, which is corrosive. Please refer to the SDS. Do not allow to contact skin or eyes. If exposed, flush with water.

Disposal:

Decontaminate materials and dispose of waste per your site practices and as required by local regulations. Do not dispose of these materials down the drain. Please note that there is a potential for mycotoxin contamination in or on any of the kit components provided.

- Dispose of all materials, containers and devices in the appropriate receptacle after use.
  Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste.
  Eject contaminated pipette tips and discard all other related materials into this container.
- Once the assay is completed, the container should be treated with a sufficient amount of 5 - 6% sodium hypochlorite (NaOCl) to saturate the contents of the container (approximately 1/10th the volume of the container). The NaOCl will denature the mycotoxins and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
- In the case of an accidental toxin spill, treat the spill surface with 5 - 6% NaOCl for a minimum of 10 minutes, followed by 5% aqueous acetone. Wipe dry with absorbent paper towels.

Preparation of Samples

Note: The sample must be collected according to the appropriate established sampling techniques.

Extraction Procedure

1. Grind a representative sample to the particle size of fine, instant coffee (95% passes through a 20-mesh screen).
2. Transfer 100 mL of distilled or deionized water to a container and add 20 g of the ground sample.
   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, then filter 5-10 mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested.
5. Dilute an aliquot of the extract 1:10 with PBS-T
6. The sample is now ready for testing.
7. The final dilution for use in calculations is **1:50**.

Assay Procedure

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay. If a single channel pipettor is used, it is recommended to run no more than a total of 16 samples and standards (2 test strips).

1. Bring all the reagents to room temperature before use. Prepare wash buffer by reconstituting the PBS-T powder packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Fill to 1 Liter with distilled or deionized water and store 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 microtiter wells in another microwell holder. Return unused wells to the foil pouch with desiccant and reseal.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Dispense 200 µL of the conjugate into each mixing well.
5. Using a new pipette tip for each, add 100 µL of each standard and prepared sample to the appropriate mixing well containing conjugate. Mix by priming pipettor at least three (3) times.
   Note: The operator must record the location of each standard and sample throughout the test.
6. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to a corresponding antibody-coated microwell. Incubate at room temperature for 15 minutes.
   Note: The mixing wells contain enough solution to run each standard and/or sample in duplicate (recommended). If running each standard or sample in singlets or if more replicates are needed, the volumes of assay diluent and sample/ standard should be scaled accordingly.
7. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-T wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 5 washes.
8. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
9. Measure the required volume of substrate reagent (1 mL/strip or 120 µL/well) and place into a separate container. Add 100 µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
10. Measure the required volume of stop solution (1 mL/strip or 120 µL/well) and place into a separate container. Add 100 µL in the same sequence and at the same pace as the substrate reagent was added.
11. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450 nm filter. Record the optical density (OD) of each microwell.
12. Setting the zero standard as 100% binding (B_o), calculate % binding (%B) for each standard and sample as a percentage of the zero binding (%B/B_o).

Interpretation of Results

Construct a dose-response curve using the OD values expressed as a percentage (%B/B_0) of the OD of the zero (0.0 ng/mL) standard against the DON content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of each standard vial refers to the contents of that vial. However, the sample has been diluted at a 1:5 ratio with distilled or deionized water in the Extraction Procedure by a further 1:10 dilution in wash buffer, so the level of DON shown by the standard must be multiplied by 50 in order to indicate the µg per gram (ppm) of the commodity as follows.

Standard (ng/mL)	Commodity* (ppm) 1:50
0.0	0.0
10.0	0.5
20.0	1.0
50.0	2.5
100.0	5.0
200.0	10.0

*Commodities here are wheat, barley, animal feed and corn.

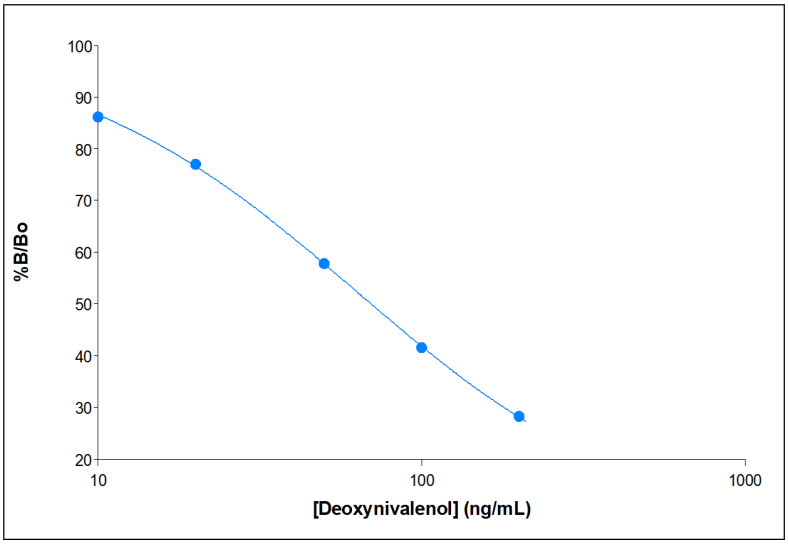
The sample dilution results in a range of detection of 1 - 20 ppb. If a sample contains DON at a greater concentration than the highest standard, it should be diluted appropriately in wash buffer (PBS-T) and retested. The extra dilution step should be taken into consideration when expressing the result.

Assay Characteristics

Data from 15 consecutive standard curves gave the following results:

Standard (ng/ml)	Concentration in Commodity (ppm) 1:50	%B/B_0	CV (%)
0.0	0.0	100.0	-
10.0	0.5	86.2	5.1
20.0	1.0	77.0	5.0
50.0	2.5	57.8	7.8
100.0	5.0	41.6	11.4
200.0	10.0	28.2	10.9

The below figure is a representative standard curve for DON based on the data table on the previous page.



Recoveries of 0.5 ppm, 2.5 ppm and 5.1 ppm from certified reference material (wheat) were as follows based on the average of three (3) independent experiments:

Wheat Reference Sample (ppm)	ppm
0.5 + 0.07	0.40
2.5 + 0.1	2.14
5.1 + 0.3	4.63

Recoveries of 2.5 ppm DON spiked into two commodities were as follows:

Type of Commodity	ppm	% Recovery
Animal feed	1.85	73.9
Corn	2.05	82.1

Technical Assistance

For questions or comments, please contact your local distributor. You can also email techsupport@hygiena.com, visit our Contact Us page for regional phone numbers or request technical support at https://www.hygiena.com/hygiena/technical-support-request.html.