DEOXYNIVALENOL (DON) ASSAY

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat. No. 941DON01M-96

DEOXYNIVALENOL (Vomitoxin)

Deoxynivalenol (DON) is a low molecular weight metabolite of the tricothecene 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

The HELICA Deoxynivalenol (DON) 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.

ASSAY PRINCIPLE

The HELICA Deoxynivalenol (DON) 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 horse-radish 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 chromagen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm (OD_{450}). 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.

REAGENTS PROVIDED

1 X Pouch	**Antibody Coated Microwell Plate**		96 wells (12 eight well strips) in a microwell holder coated with a mouse anti-deoxynivalenol monoclonal antibody, *Ready-to-Use.*
1 X Plate	**Mixing Wells**	Green	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-to-Use.*
6 X Vial	**Deoxynivalenol Standards**	Black Cap	1.5mL/vial of deoxynivalenol at the following concentrations: 0.0, 10.0, 20.0, 50.0, 100.0, and 200.0ng/mL in deionized water, *Ready-to-Use.*
2 X Bottle	**DON HRP-Conjugate**	Green Cap	2 x 12mL of deoxynivalenol conjugated to peroxidase in buffer with preservative, *Ready-to-Use.*
1 X Bottle	**Substrate Reagent**	Blue Cap	12mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use.*
1 X Bottle	**Stop Solution**	Red Cap	12mL Acidic Solution, *Ready-to-Use.*
1 X Pouch	**Washing Buffer**		PBS with 0.05% Tween20, bring to 1 liter with distilled water and store refrigerated.

MATERIALS REQUIRED BUT NOT PROVIDED

Extraction Procedure

- Grinder sufficient to render sample to particle size of fine instant coffee
- Collection Container: Minimum 125mL capacity
- Balance: 20g measuring capability
- Graduated cylinder: 100mL
- Distilled or deionized water: 100mL per sample
- Filter Paper: Whatman #1 or equivalent
- Filter Funnel

Assay Procedure

- Pipettor with tips: 100µL and 200µL
- Timer
- Wash bottle
- Absorbent paper towels
- Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19° - 27°C) before use.
2. Store reagents at 2°-8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not use solutions if cloudy or precipitate is present.
4. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
5. Adhere to all time and temperature conditions stated in the procedure.
6. Samples tested should have a pH of 7.0 (±1.0). Excessive alkaline or acidic conditions may affect test results.
7. Never pipette reagents or samples by mouth.
8. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
9. 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.
10. Dispose of all materials, containers and devices in the appropriate receptacle after use.
11. HRP-labeled conjugate and TMB-substrates are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

KIT SAFETY AND WASTE DISPOSAL INSTRUCTIONS

Mycotoxins (aflatoxins, trichothecenes, and others) are well known carcinogens in humans and are thus considered highly toxic. 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. It is strongly advised that gloves, a lab coat, and safety glasses be worn at all times while handling mycotoxin kits and their respective components.

1. Before conducting the assay, prepare a waste container to act as a receptacle for your kit waste. Eject contaminated pipette tips and all other related materials into this container.
2. 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). 5-6% NaOCl will denature the mycotoxins and neutralize the waste, making it safe for disposal. Invert the container several times to thoroughly coat all waste.
3. 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.

EXTRACTION PROCEDURE

1. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen).
2. Transfer 100mL of distilled or deionized water to a container and add 20g 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 - 10mL 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 wash buffer.
6. The sample is now ready. The standards require no pre-dilution before use.
7. Final dilution for use in calculation = 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 that no more than a total of 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container.
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.
3. Dispense 200µL of the Conjugate into each mixing well.
4. Using a new pipette tip for each, add 100µL of each standard and prepared sample to the appropriate mixing well containing Conjugate. Mix by priming pipettor at least 3 times.
   Note: Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100µL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 15 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS Tween 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 (1mL/strip or 120µL/well) and place in a separate container. Add 100µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
9. Measure the required volume of Stop Solution (1mL/strip or 120µL/well) and place in a separate container. Add 100µL in the same sequence and at the same pace as the Substrate Reagent was added.
10. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.
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).

INTERPRETATION OF RESULTS (SEE EXTRACTION PROCEDURE FOR SPECIFIC APPLICATIONS)

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/Bo) 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 contained on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted at a 5:1 ratio with distilled or deionized water in the EXTRACTION PROCEDURE followed by a further 10:1 dilution in wash buffer, and 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:

	Wheat, barley, animal feed, and corn
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

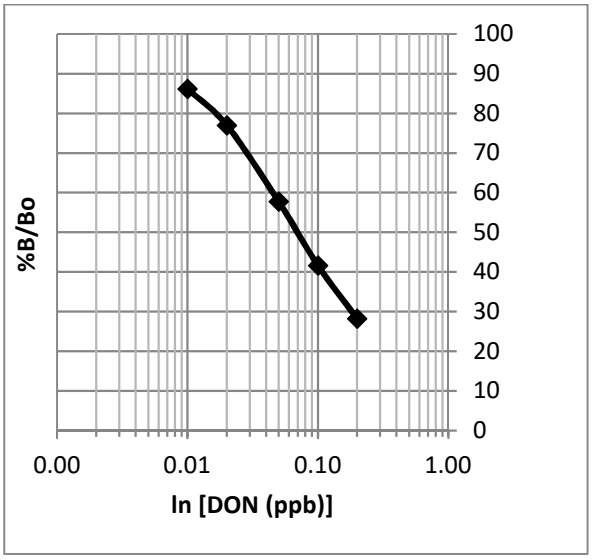
The sample dilution results in a standard curve from 0.5-10.0ppm. If a sample contains DON at greater concentration than the highest standard, it should be diluted appropriately in wash buffer and retested. The extra dilution step should be taken into consideration when expressing the result.

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 0.5ppm, 2.5ppm, and 5.1ppm from certified reference material (wheat) were as follows based on three 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 2.5ppm DON spiked into two commodities were as follows:

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

Correlation studies were also completed to compare the performance between HELICA's Deoxynivalenol (DON) Assay and HPLC determination. The figures on the following page show that there is an excellent correlation between HELICA's DON Assay and HPLC over a range of <0.7 to >1.8ppm for wheat samples and <0.6 to >3.4ppm for barley samples.