DEOXYNIVALENOL (DON) ASSAY

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.

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 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 and color (blue) develops. 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 450nm (OD_{450}). The optical densities of the samples are compared to the OD's of the kit standards and an interpretative result is determined.

MATERIALS SUPPLIED

1 pouch: **Antibody coated microwells**, 96 wells (12 eight well strips) in a microwell holder coated with a mouse anti-deoxynivalenol antibody
1 plate: **Dilution wells (green)**, 96 non-coated wells (12 eight well strips) in a microwell holder
6 vials: **DON Standards** 1.5ml/vial of deoxynivalenol at 0, 10, 20, 50, 100, and 200 ng/mL
2 bottles: **DON HRP-conjugate** 2 x 12ml of deoxynivalenol conjugated to peroxidase in buffer with preservative
1 bottle: **Substrate Reagent**, 15ml stabilized tetramethylbenzidine (TMB)
1 bottle: **Stop Solution,** 15ml Acidic Solution

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 (19º - 27ºC) before use.
2. Store reagents at 2 to 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 effect the 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.

EXTRACTION PROCEDURE
Note: The sample must be collected according to established sampling techniques

1. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Weigh out a 20g ground portion of the sample and add 100ml of distilled or deionized water.
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 the sample 1:10 by adding 1ml of sample extract to 9ml of distilled or deionized water.
6. The sample, at a final dilution of 1:50, is now ready for testing.

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.
2. Place one Dilution 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 Dilution Well.
4. Using a new pipette tip for each, add 100µl of each Standard and Sample to appropriate Dilution 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 Dilution Well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 15 minutes.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with distilled or deionized water, then decanting the water 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 water.
8. Measure the required volume of Substrate Reagent (1 ml/strip or 120 µl/well) and place in a separate container. Add 100µl to each microwell. Incubate at room temperature for 5 minutes.

9. Measure the required volume of Stop Solution (1 ml/strip or 120 µl/well) and place in a separate container. Add 100µl in the same sequence and at the same pace as the Substrate 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.

INTERPRETATION OF 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 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 water followed by a further 10:1 dilution, and so the level of DON shown by the standard must be multiplied by 50 in order to indicate the ug of DON 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.

APPENDIX

PERFORMANCE DATA

WITHIN ASSAY VARIATION

A typical example of the Helica DON assay run in duplicate yielded the following standard curve and within assay variation.

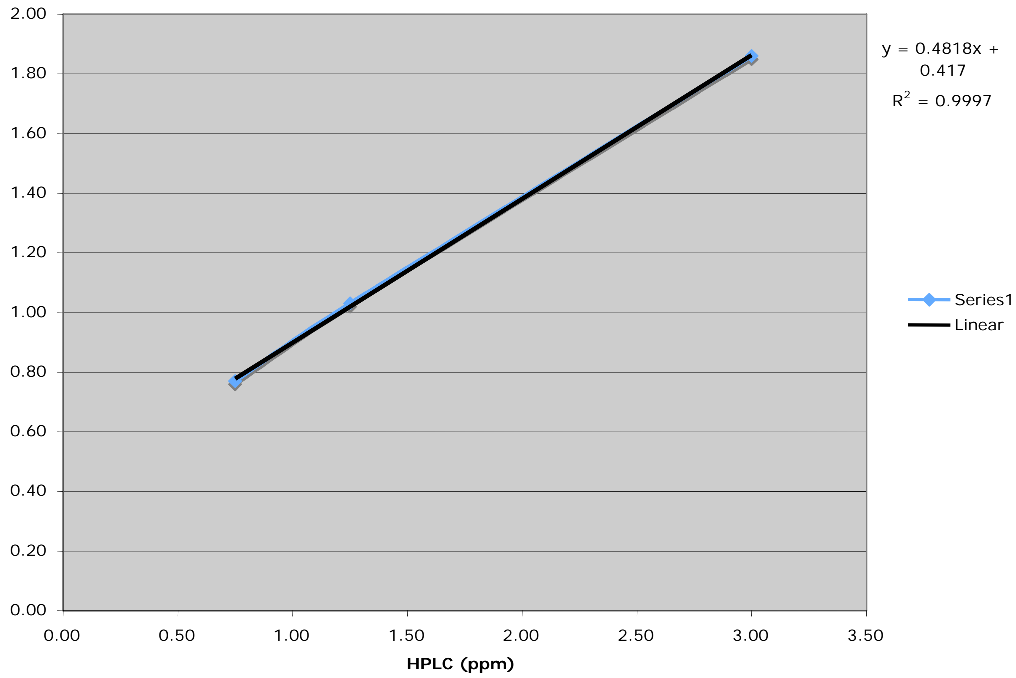
ppm in sample	Mean OD	CV%
0.0	2.110	1.8
0.1	1.793	1.0
0.2	1.616	1.9
0.5	1.262	1.1
1.0	0.985	1.0
2.0	0.655	1.5

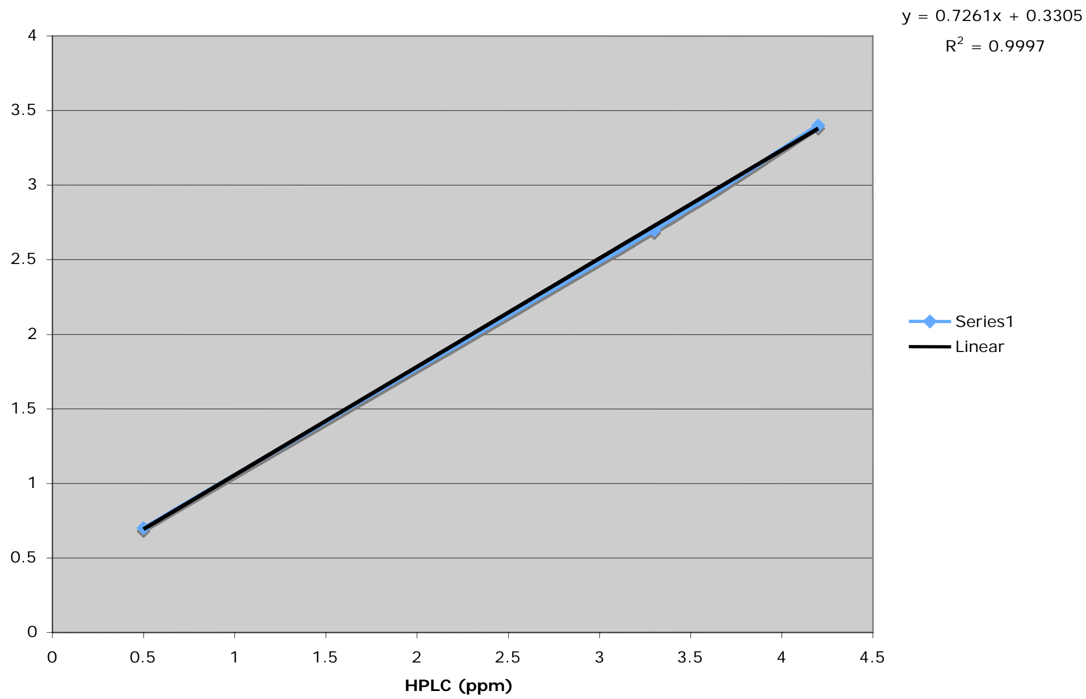
BETWEEN ASSAY VARIATION

Between assay variation is expressed as percentage of Bo for each standard.
n= 6 assays.

ppm in sample	B/Bo%	CV%
0.1	83.3	1.6
0.2	74.6	2.3
0.5	57.1	4.6
1.0	42.8	6.5
2.0	29.5	4.4

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 due to ‘matrix inhibition’ effects, it follows that the LOD is commodity specific and should be measured empirically for each different commodity.
Using the Helica DON assay: LOD for wheat is 0.15 ppm   n= 10


DON Helica vs HPLC (Wheat)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
HPLC (ppm)
y = 0.4818x + 0.417
R2 = 0.9997
Series1
Linear


DON Helica vs HPLC (barley)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
HPLC (ppm)
y = 0.7261x + 0.3305
R2 = 0.9997
Series1
Linear