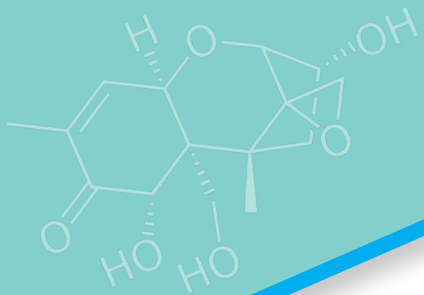

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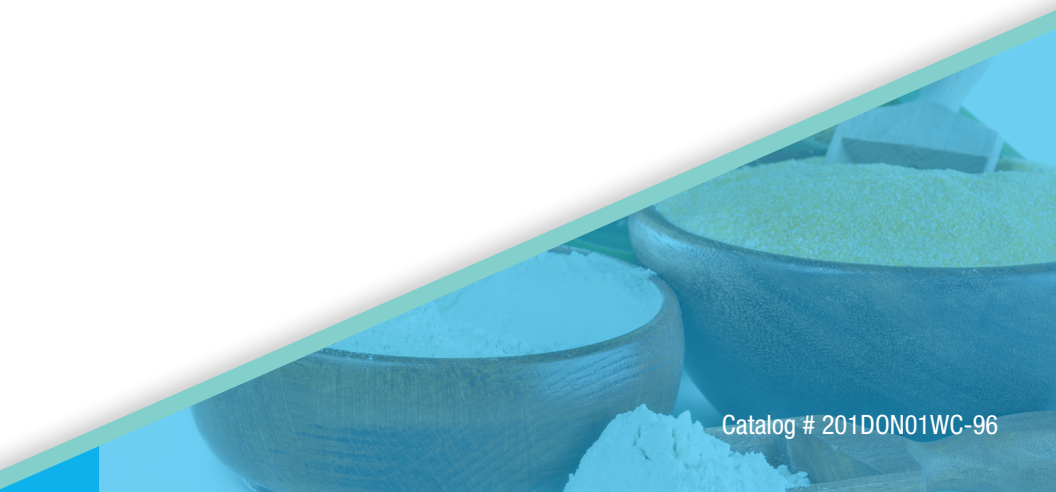
Helica™ Deoxynivalenol RAPID ELISA

For the quantitative detection of deoxynivalenol in corn and wheat.



Catalog # 201DON01WC-96

Helica™ Deoxynivalenol RAPID ELISA

For the quantitative detection of deoxynivalenol in corn and wheat.

This package insert must be read in its entirety before using this product.

Contents

Intended Use

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. The Helica™ Deoxynivalenol RAPID ELISA kit was developed to determine deoxynivalenol with a wide range of 0.5 - 30 ppm in corn and wheat using aqueous extraction procedure and certified by the USDA Federal Grain Inspection Service (FGIS) for the quantitative determination of deoxynivalenol (Certificate No. FGIS 2021-145).

Field of Use

Data obtained from Helica™ assays should not be used for human diagnostic or human treatment purpose. Assays are not approved by the United States Food and Drug Administration or any other U.S. or non-U.S. 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 supervision of technically qualified persons.

Principle of the Method

The Helica™ Deoxynivalenol RAPID ELISA is a direct competitive enzyme immunoassay. A deoxynivalenol (DON) specific antibody is coated to a polystyrene microwell. Toxins are extracted from a sample with 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 an 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 450nm (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.

Storage and Shelf Life

- Bring all reagents to room temperature (18 - 30°C) before use.
- Store reagents at 2°C to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
- Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.

Precautions

Read this manual carefully before starting the test. The test must be performed by specialized and trained staff.

- Handle the test kit in accordance with good laboratory practices (GLP).
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond the expiration date of the kit. The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate.
- Substrate solution is light sensitive. Avoid exposure to direct light.
- Do not allow wells to dry completely.
- Handle any solution with gloves.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- Substrate solution contains TMB, which is highly toxic if inhaled, ingested, or comes in contact with the skin. Please refer to the SDS.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water. Please refer to the SDS.
- Stop Solution contains phosphoric acid, which is corrosive. Please refer to the SDS.
- Avoid incubating on cold work benches.

Kit Contents

1X Pouch	Antibody coated microwell plate		96 wells (12 eight well holder) coated with a mouse anti-deoxynivalenol monoclonal antibody, *Ready-to-Use*.
1X Plate	Mixing wells	Green	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-to-Use.*
6X Vials	Deoxynivalenol standards	Black cap	1.0 mL/vial of deoxynivalenol at the following concentrations: 0.0, 0.01, 0.025, 0.05, 0.1, and 0.2 µg/mL in aqueous solution, *Ready-to-Use*.
2X Bottles	Deoxynivalenol-HRP Conjugate	Green cap	2 x 12 mL of deoxynivalenol conjugated peroxidase in buffer with preservative, *Ready-to-Use*.
1X Bottle	Substrate reagent	Blue cap	12 mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use*.
1X Bottle	Stop solution	Red cap	12 mL acidic solution, *Ready-to-Use*.
1X Pouch	Washing buffer		PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.

Materials Required But Not Provided

- Single or multi-channel pipettor with 100, 200, and 1000 µL tips
- Timer
- Wash bottle
- Absorbent paper towels
- Microcentrifuge and tubes
- Microtubes
- Analytical balance
- Graduated cylinders (500 mL and 1000 mL)
- Extraction container
- Whirl-Pak Stand-up bag
- Reagent boat
- Kimwipe
- Vortex mixer
- Distilled (or deionized) water
- Water bath
- Microplate reader equipped with a 450 nm filter (BioTek 800TS)

Preparation of Sample

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

1. Place a bottle containing deionized or distilled water in water bath set at 30 °C.
2. Let it pre-warm for 1 hour before use.
3. Weigh 5 grams ground sample into a clean container.
4. Add 50 mL of warm deionized or distilled water and close securely. **USDA/FGIS Method: Weigh 50 ± 0.2 grams and add 500 mL into a Whirl-Pak bag.**
5. Shake by hand for a few seconds to suspend sample in water.
6. Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for three minutes.

Preparation of Sample, con't.

7. Carefully place the sample on the bench. Do not shake or swirl.
8. Transfer 1 mL of the extract into a microcentrifuge tube.
9. Centrifuge at 6000 rpm for 1 minute.
10. The supernatant is the sample extract to be used in the Extract Dilution Procedure below and can be used for the next one hour.

Extract Dilution Procedure

Note: Two different dilution procedures are needed to cover the full conformance range. Each diluted extract is for single use only. Do not re-use for the re-test.

1. **Diluted Extract A (for the 0.5 – 5.0 ppm quantitation range)**

   Pour the needed volume of Wash Buffer Solution into the reagent boat. Using a 30 - 300 µL variable volume multichannel pipettor, transfer 200 µL of Wash Buffer Solution into the microtubes. Using a 20 - 200 µL variable volume single channel pipettor, transfer 100 µL of the sample extract into the microtube containing 200 µL of Wash Buffer Solution. This is Diluted Extract A. Vortex for a few seconds prior to use. Repeat this process for the remaining sample extracts.

2. **Diluted Extract B (for the 5.0 – 30 ppm quantitation range)**

   Using a 30 - 300 µL variable volume multichannel pipettor, transfer 50 µL of the Diluted Extract A into the microtube containing 450 µL (measured using a 100 -1000 µL variable volume single channel pipettor) of Wash Buffer Solution. This is Diluted Extract B. Vortex for a few seconds prior to use.

Assay Procedure

Note: In addition to the six standards, one can analyze at the most sixteen samples (two strips of wells, total 16 wells) per run. For unknown samples, Diluted Extract A should be tested first. If the results are above 5.0 ppm, Diluted Extract B should be prepared and analyzed.

1. Bring all reagents and samples to room temperature (18 – 30 °C / 64 – 86 °F) before use and perform the sample preparation at room temperature.
2. Remove one green-marked mixing well for each sample and another six green-marked mixing wells for six standards.

Note: Use two wells per sample if higher quantitation range (5.0 to 30 ppm) is also tested at the same time.

3. Remove an equal number of antibody-coated wells and return unused wells to the foil pack with desiccant.
4. Mix each reagent by swirling the reagent bottle prior to use.
5. Using a 20 - 200 µL variable volume single channel pipettor, dispense 200 µL of each standard into the microtube.
6. Pour the needed volume of conjugate from the green-capped bottle into the reagent boat. Using a 30 – 300 µL variable volume multichannel pipettor, dispense 150 µL of conjugate into each green-marked mixing well.
7. Using a 30 – 300 µL variable volume multichannel pipettor, add 50 µL of standards and samples to the corresponding green-marked mixing wells. Mix by pipetting up and down 20 times.

   Note: For 0.50 – 5.0 ppm quantitation range, use 50 µL of Diluted Extract A. For 5.0 – 30 ppm quantitation range, use 50 µL of Diluted Extract B.
8. Using a 30 – 300 µL variable volume multichannel pipettor, transfer 100 µL (from step 7) into the antibody-coated wells. Incubate at room temperature for 4 minutes.
9. Discard the contents from the wells into a discard basin. Using a wash bottle, fill the wells with Wash Buffer Solution and dump the solution out of the wells into a discard basin. Repeat this step four more times.
10. Tap the wells (face down) on a layer of absorbent towels to remove residual buffer.
11. Pour the needed volume of substrate reagent from the blue-capped bottle into the reagent boat. Using a 30 – 300 µL variable volume multichannel pipettor, add 100 µL of substrate reagent into each well and cover to avoid direct light. Incubate at room temperature for 4 minutes.
12. Pour the needed volume of stop solution from the red-capped bottle into the reagent boat. Using a 30 – 300 µL variable volume multichannel pipettor, add 100 µL of stop solution into each well in the same sequence and pace as the substrate reagent was added.
13. Wipe the bottom of the wells with a lint-free Kimwipe and remove air bubbles, if there are any, using a pipette tip.
14. Read the optical density (OD) at 450 nm using the BioTek 800 TS reader. Read within 10 minutes after addition of stop solution.

Interpretation of Results

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 deoxynivalenol 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 1:30 ratio for Diluted Extract A and 1:300 ratio for Diluted Extract B, and so the level of deoxynivalenol shown by the standard must be multiplied by 30 and 300 in order to indicate the µg of deoxynivalenol per gram of commodity (ppm) as follows:

Standard (µg/mL)	Diluted Extract A (1:30 dilution) Quantitation range of 0.5 – 5 ppm	Diluted Extract B (1:300 dilution) Quantitation range of 5 – 30 ppm
0	0	0
0.01	0.3	3
0.025	0.75	7.5
0.05	1.5	15
0.1	3	30
0.2	6	60

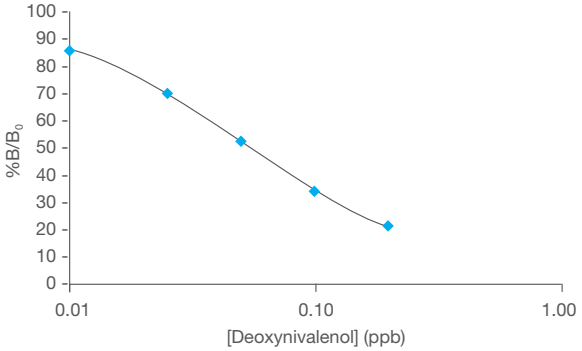
For Diluted Extract A, results are valid in the range of 0.5 to 5 ppm. If the result is above 5 ppm, re-test with Diluted Extract B.

Assay Characteristics

Data from seven consecutive standard curves gave the following results.

Standard (ng/mL)	$\%B/B_0$	CV (%)
0	100	-
0.01	86	3.11
0.025	70	5.45
0.05	52	3.45
0.1	34	6.34
0.2	21	3.64

The graph below represents the data in the table above.



Accuracy

Accuracy of the assay with corn and wheat samples naturally contaminated with deoxynivalenol.
(n = 21 per each contamination level)

Deoxynivalenol in sample (ppm)	Corn (ppm)	USDA Criteria (ppm)	Deoxynivalenol in sample (ppm)	Wheat (ppm)	USDA Criteria (ppm)
0.5	0.50	0.3 – 0.7	0.5	0.47	0.3 – 0.7
1.7	1.86	1.3 – 2.1	1.9	1.95	1.4 – 2.4
4.9	4.93	3.9 – 5.9	5.4	5.01	4.3 – 6.5
27.6	28.21	22.1 – 33.1	34.4	34.52	27.5 – 41.3

Technical assistance:

For questions or comments, please contact your local distributor. You can call 1-714-578-7830 or email Helica-ts@hygiena.com.

Technical support can also be requested at:
https://www.hygiena.com/hygiena/technical-support-request-helica.html

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