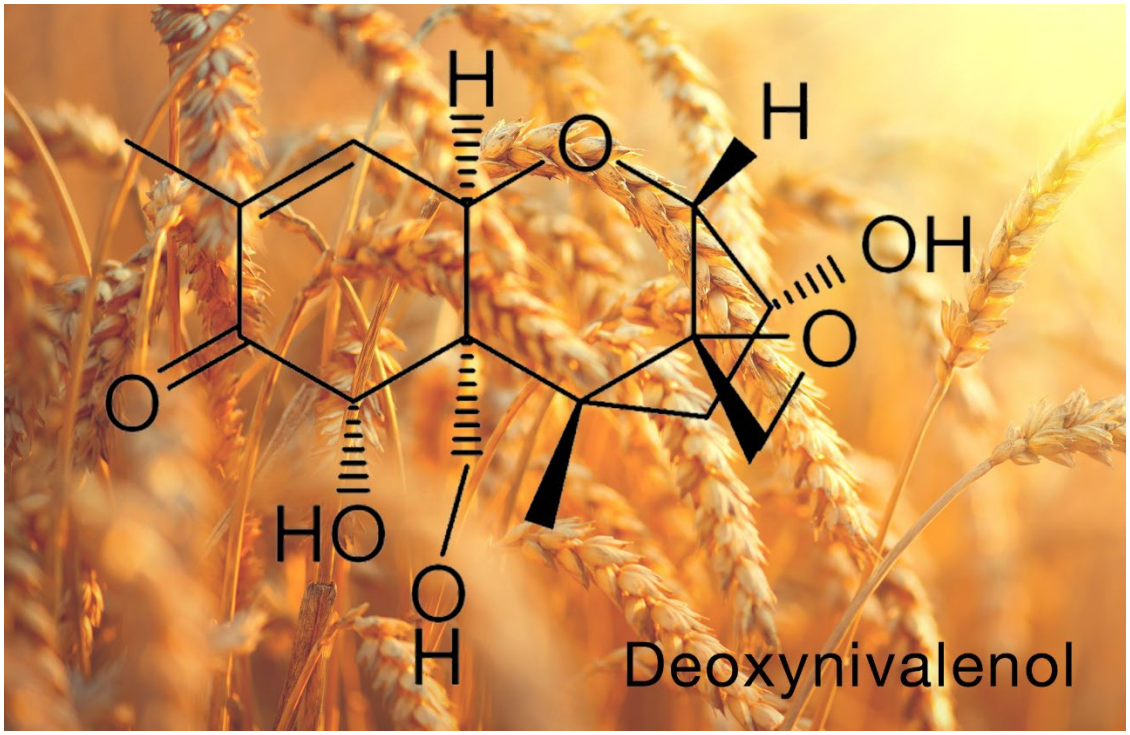
# Helica® Deoxynivalenol Rapid ELISA

**Product Number – KIT5011 (201DON01WC-96)**



# Helica® Deoxynivalenol Rapid ELISA

***For the quantitative detection of deoxynivalenol in corn and wheat.***

## Table of Contents

## Introduction – Deoxynivalenol

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. 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 an aqueous extraction procedure and is certified by the USDA Federal Grain Inspection Service (FGIS) for the quantitative determination of deoxynivalenol (Certificate No. FGIS 2021-145).

## 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 Rapid ELISA assay is a direct competitive enzyme immunoassay for the quantitative detection of deoxynivalenol in corn and wheat.

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 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 assay 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 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 monoclonal antibody, *Ready-to-Use*. |
| 1X Pouch | Dilution plate | 96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-to-Use*. |
| 6X Vials | Standards | 1.0 mL/vial of Deoxynivalenol at the following concentrations: 0.0, 10, 25, 50, 100 and 200 ng/mL in aqueous solution, *Ready-to-Use*. |
| 2X Bottles | Conjugate | 2 x 12 mL of peroxidase-conjugated Deoxynivalenol 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. |

*TWEEN® 20 is a registered trademark of CRODA International Plc.*

## Materials Required but Not Provided

- Variable single and multichannel pipettors with tips: 100, 200 and 1000 µL
- Distilled or deionized water
- Microtubes
- Timer
- Wash bottle
- Absorbent paper towels
- Whatman #1 filter paper and funnel
- Centrifuge
- Microcentrifuge and tubes
- Analytical balance
- Vortex mixer
- Blender
- Distilled water or deionized water
- Graduated cylinders: 500 and 1000 mL
- Extraction container
- Whirl-Pak stand-up bag
- Reagent boat
- Kimwipes or similar lint-free wipe
- Methanol, reagent grade
- 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.

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

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. Final dilution is 1:300.

## Assay Procedure

*Note: In addition to the six standards, one can analyze, at 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 used to prepare and analyze samples.*

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. Perform sample preparation at room temperature.

2. Place one mixing well in a microwell holder for each sample to be tested Place another six (6) wells in the microwell holder for six (6) standards. Remove an equal number of antibody-coated wells and return unused wells to the foil pack with desiccant and reseal.
   *Note: Use two wells per sample if a higher quantitation range (5.0 to 30 ppm) is tested at the same time.*
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Using a 20 - 200 µL variable volume single channel pipettor, dispense 200 µL of each standard into the microtube.
5. Transfer 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.
6. Using a 30 - 300 µL variable volume multichannel pipettor, add 50 µL of standards and samples to the corresponding 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.*
7. 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.
8. 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.
9. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
10. Transfer 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.
11. Transfer 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.
12. Read the optical density (OD) at 450 nm using a plate reader. Read within 10 minutes after the addition of stop solution.

## Interpretation of Results

Construct a dose-response curve using the OD values expressed as a percentage of the OD of the zero (0.0 µg/ml) 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 a 1:300 ratio for Diluted Extract B, 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 (ng/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 |
| 10 | 0.3 | 3 |
| 25 | 0.75 | 7.5 |
| 50 | 1.5 | 15 |
| 100 | 3 | 30 |
| 200 | 6 | 60 |

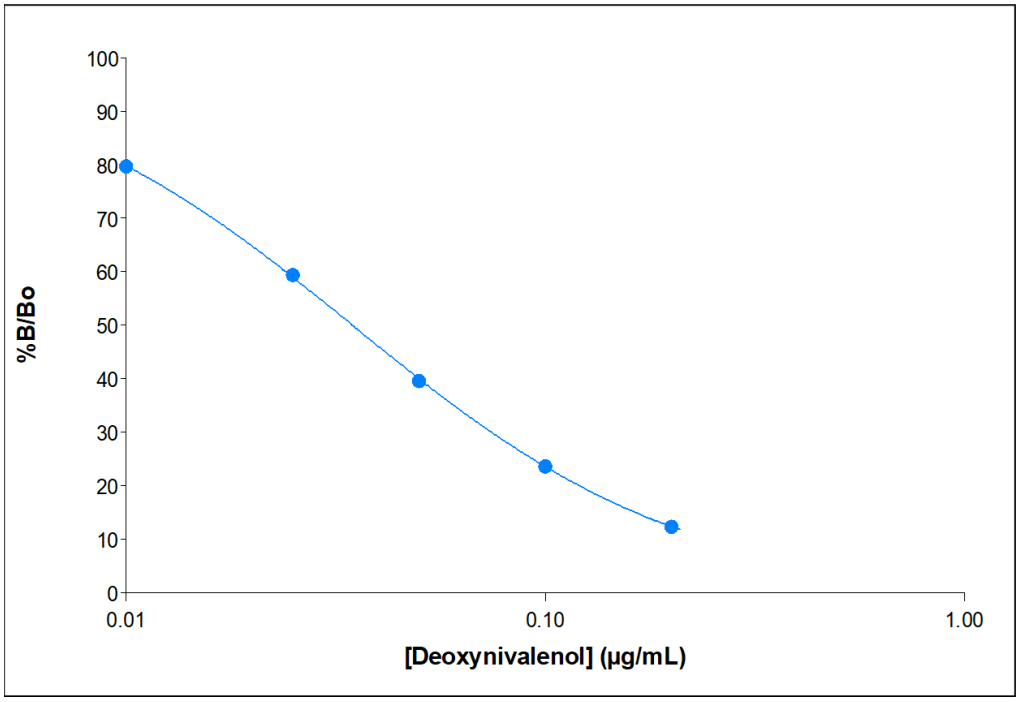
For Diluted Extract A, results are valid in the range of 0.5 to 5 ppm. If the result is above 5 ppm, re-test with Diluted Extract B.

## Assay Characteristics

Data from the average of seven (7) consecutive standard curves gave the following results.

| Standard (µg/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

The accuracy of the assay with corn and wheat samples naturally contaminated with deoxynivalenol is shown below (n=21 per each contamination level).

| Deoxynivalenol in Sample (µg/mL) | Corn (ug/mL, ppm) | USDA Criteria (µg/mL, ppm) | Deoxynivalenol in Sample (µg/mL, ppm) | Wheat (µg/mL, ppm) | USDA Criteria (µg/mL, 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 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.