# Deoxynivalenol(DON) ELISA Kit

**Catalog No. CSB-E12829f**

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **DON** concentrations in wheat, corn and feeding stuff.
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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## INTRODUCTION

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.

## TEST PRINCIPLE

This kit is a competitive enzyme-linked immunoassay intended for the quantitative detection of DON in cereal grains and other commodities including animal feeds.

## DETECTION RANGE

0.5 ppm -4.0 ppm. The standard curve concentrations used for the ELISA's were 4.0 ppm, 2.0 ppm, 1.0 ppm, 0.5 ppm.

## SENSITIVITY

The minimum detectable dose of DON is typically less than 0.2ppm.
For wheat, corn and feeding stuff samples:
Recovery≥80%
Inter-assay CV%＜15%
Intra-assay CV%＜15%

## MATERIALS PROVIDED

| Reagent | Quantity |
|---|---|
| Assay plate | 1 |
| Standard | 5×1ml |
| Antibody | 1 x 6 ml |
| HRP-conjugate | 1 x 12 ml |
| Chromogen | 1 x 12 ml |
| Wash Buffer | 1 x 20 ml (20×concentrate) |
| Stop Solution | 1 x 6 ml |

| Standard | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| ng/ml | 0 | 50 | 100 | 200 | 400 |

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- An incubator which can provide stable incubation conditions up to 37℃±0.5℃.

## STORAGE

1. The kits should be stored at 2-8°C **(Never freeze kit components.)** upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

## SAMPLE PREPARE

### 1. Wheat, corn and rice

Weigh 5g of ground sample and combine with 25 ml of dd$H_2O$ in a clean container with tight fitting lid. Vigorously shake the container for 3 minutes. Filter using qualitative filter paper and collect the extract into a clean container. Weigh 4 ml Extract, add 4 ml dd$H_2O$, mix well. Filter using qualitative filter paper.

### 2. Beer, malted milk juice

Weigh 5ml sample and combine with 45 ml of dd$H_2O$ in a clean container with tight fitting lid. Mix well. Filter using qualitative filter paper.

***Note: Assay immediately or store samples at 2-8℃ i n brown glass bottles.***

## REAGENT PREPARATION

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 400 ml of Wash Buffer.

## ASSAY PROCEDURE

Note:

- **Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.**
- **Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps.**

1. Set a Blank well, add 100μl Wash Buffer (Don't add Antibody). Then add 50μl of Standard or Sample to other wells, and add 50μl Antibody, mix well. Cover with the adhesive strip. Incubate for 30 minutes at 37°C.
   It is recommend to complete this step in a short time.
2. Aspirate each well and wash, repeating the process four times for a total of four washes. Wash by filling each well with Wash Buffer (200μl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step

is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 100μl of HRP-conjugate to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at 37℃.
4. Repeat the aspiration and wash four times as before.
5. Add 100μl of Chromogen to each well. Incubate for 5-10 minutes at 37℃. Keeping the plate away from drafts and oth er temperature fluctuations in the dark.
6. Add 50μl of Stop Solution to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

***Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.***

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the

mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the DON concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate solution and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.