VERSION N2
CAT.NUMBER: D4548

1 STANDARD DON

ELISA TEST | In vitro analysis

for the quantitative determination of Deoxynivalenol in grains, cereals and animal feed

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

1 Standard DON, D4548, is an immunoassay method that determines the Deoxynivalenol, in grains, cereals and other commodities including animal feed. The ELISA kit contains all reagents required for the immunoassay method. The ELISA test is adequate for 48 definitions (Zero Standard (St1) is included). A spectrophotometer for microtiter ELISA plate is required.

Matrices:

Cereals: Alfalfa, Barley, Biscuits, Bran sticks, Bread, Brown rice, Corn, Corn flakes, Corn flour, Corn Germ, Corn Gluten, Corn Gluten Meal, Cottonseed, DDGS, Dried sunflower, Oat bran, Oat flakes, Oats, Pasta, Pet food, Popcorn, Raw Rye, Rice, Rye flour, Sesame, Silage, Sorghum, Soy beans, Soy flour, Wheat, Wheat bran, Wheat flour.

Other: Cocoa, Coconut residue, Milk

- Sample preparation: extraction
- Test time (incubation time after samples and reagents preparation): 5min
- Standard curve range: 0 - 5ppm
- Shelf life: 12 months
- Storage: 2-8^{o}C

This is an electronic version, please verify always the last one included in the kit.

Specifications

- The LOD of the method is 0.07 ppm DON.
- The LOQ of the method is 0.10 ppm DON.
- The recovery of spiked extractions-matrices was 101.5% (CV = 11.7%).
- IC50 = 0.3 - 1.1 ppm
- The cross-reaction of the anti-DON antibody with 15-acetyl-DON, DON 3-acetyl-DON, Nivalenol and Patulin is >100, 100, <0.1, <1 and <0.1%, respectively.

1. Description

1 Standard DON is an ELISA test for the detection of Deoxynivalenol in grains, cereals and animal feed.

2. General Information

Deoxynivalenol (DON), also known as vomitoxin, is a member of the trichothecene mycotoxins produced by fungi of the Fusarium genus (*F. graminearum*). Grains including barley, wheat, oats, corn and maize are frequently infected by this fungus. Deoxynivalenol, along with 3-acetyl- and 15 -acetyl-DON, constitutes a highly toxic molecule and it is considered to play a crucial role in immunological and nervous system problems. Due to their cytotoxicity, these toxins will always be a risk to human and animal health. Most controlling government agencies worldwide have regulations regarding the amount of DON allowable in human and animal foodstuffs. Accurate and rapid determination of DON presence in commodities is of paramount importance.

3. Principle of the Method

The quantitative test is based on the enzyme linked immunosorbent assay principles. The wells of the microtiter strips are coated with DON specific antibodies. Toxins are extracted from a ground sample with 70% methanol. Deoxynivalenol standards or samples and DON-HRP conjugate (detection solution) are added into the coated wells. DON-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by DON of standards or samples. Any unbound DON-HRP conjugate of detection solution is removed in a washing step. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the detection antibody. The color development is then stopped by the addition of acid turning the resultant final product yellow. The measurement is made photometrically at 450 nm and the intensity of the produced colored complex is indirectly proportional to the concentration of DON present in the samples and standards.

4. Reagents Provided

1 Standard DON ELISA kit contains sufficient reagents and materials for 48 measurements (including Zero Standard test). Zero Standard (St1) is the only standard provided and the B/Bo values of St2-St5 (0.1 - 5 ppm) are reported in the Quality Assurance Certificate of each lot.

Reagents (Store at 2-8ºC)	Quantity for 48 wells	State	Vial cap color
Single-Break Strip Plate	48 wells	Ready to use (precoated)	-
Dilution Microwells	48 wells	Ready to use (green color)	-
Zero Standard (St1) (0 ppm of DON in organic solution)	1 plastic vial (3ml)	Ready to use	Brown
1 Standard DON Detection Solution	1 plastic vial (6ml)	Ready to use	Green
Wash Buffer	1 plastic vial (50ml)	20X Concentrate (dilute in distilled water)	White
TMB Substrate	1 plastic vial (6ml)	Ready to use	Brown
Stop Solution	1 plastic vial (6ml)	Ready to use	White

5. Materials required but not provided

- A grinder sufficient to render sample to particle size of fine instant coffee
- Balance with 0 - 50 g measuring capability and Graduated cylinder - 100 mL
- Methanol (70 mL reagent grade per sample) and Distilled or deionized water
- Filter Paper Whatman #1 or equivalent, Filter Funnel and Miscellaneous laboratory plastic or glass tubes 50 - 125 mL
- Vortex mixer and Microtiter plate reader fi ted with 450 nm filter
- 100, 200 and 1000 µL adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100µL is acceptable for the steps of TMB and Stop Solution)
- 50 - 300 µL multi-channel micropipette with disposable tips and reservoirs

6. Storage Instructions

Store kit reagents between 2 and 8°C (35 - 46°F). Do not freeze any components provided. Reseal immediately the unused strips of the microtiter plate in the bag together **with the desiccant bag** provided and store at 2 - 8°C. After use remaining reagents should be returned to cold storage (2 - 8°C). Expiry of the kit and reagents is stated on the labels respectively and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly as well as if the reagent is not contaminated by the first handling, in case of repeated use of one component. Because of the colorless TMB Substrate and standards 1-7 light sensitivity, avoid the exposure to direct light. Do not interchange individual reagents between kits of different lot numbers.

7. Safety and Precautions for use

- Avoid any skin contact with Stop Solution (8% H_3PO_4) and TMB (toxic). **Use gloves.** In case of contact, wash thoroughly with water.
- All reagents should be warmed in room temperature before use and covered when not in use. **Use a clean disposable plastic pipette tip for each reagent, in order to avoid cross contamination. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.**
- Use a clean plastic container to prepare the wash buffer and all residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper into the well. Read the absorbance within 60 minutes after completion of the assay.

8. Indication of corruption of kit reagents

- The bluish coloration of the chromogen substrate before the ELISA test.
- A value of less than 0.7 absorbance units (ABS 450nm) for the Standard 1 (St1).

9. Sample and reagents preparation

9.1 Reagents preparation

- Prepare the Extraction Solution (70% Methanol) by adding 30ml of distilled or deionized water to 70ml of methanol (reagent grade) for each sample to be tested.
- Dilute the 20X solution concentrate 20 fold with distilled water to give a **1X** working solution.

> **Preparation of Wash Buffer 1X:** In case of the occurrence of crystals in the Wash Buffer, the warming by gentle dismantling (using hands) of the crystals is needed. Pour entire content of the solution concentrate (50ml) into a clean 1000ml graduated cylinder, rinse the vial with distilled or deionized water and pour the content again into the cylinder and fill to a final volume of 1000ml with distilled or deionized water. Mix gently to avoid foaming, transferring the final solution from cylinder to a clean bottle and back two times. The clean bottle with **1X Wash Buffer** working solution can be left out of the refrigerator during the method procedure and subsequent be stored 2 - 8°C for one month.

9.2 Samples preparation

9.2.1 Ground samples

1. The sample must be collected according to established sampling techniques. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Weigh out a 20 g ground portion of the sample and add 100 mL of the Extraction Solvent (70% methanol) and mix in a blender for a minimum of 2 minutes. **The ratio of sample to extraction solvent is 1:5 (w/v).**
3. Allow the particulate matter to settle, filter 5 - 10 mL of the extract through a Whatman #1 filter paper (or equivalent), collect the filtrate and dilute 5 times with deionized water (example: 1 mL filtrate + 4 mL deionized water). **The ratio of sample to 14% methanol is 1:25 (w/v).**
4. Use 100 µL of each final diluted filtrate directly in the immunoassay.

NOTE 1: The extracted sample should have pH value of 6.2 - 7.5. If the pH is less than 6.2, it should be neutralized using NaOH.

NOTE 2: In case the user make an additional dilution 1:1 of filtrate with 14% methanol the range of quantification becomes 0 - 10ppm. So, use also 100µl of each diluted filtrate directly in the immunoassay and multiply the final DON ppm result x 2.

9.2.2 Milk Samples

After centrifugation at 3000xg at 4°C for 10 min, remove the upper fat layer, dilute the defatted milk sample 5 times with 17.5% methanol (**1ml of milk + 4ml of 17.5% methanol**) and vortex. Use 100µl of each diluted milk sample directly in the immunoassay and divide the final DON ppm result by 5. The LOD is 0.014ppm and the LOQ is 0.02ppm.

10. Method Procedure

10.1 Assay Design: Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for zero standard. Considering that each sample and standard can be tested in single or in duplicate, create a layout.

NOTE: It is preferred to use no more than 32 wells (four strips) in each assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	St1											
B												
C												
D												
E												
F												
G												
H												

Example plate layout

10.2 Bring all reagents to room temperature (19 - 24°C) before use. Remove the **Zero Standard** (St1) and place the **appropriate number of dilution wells** into the holder of microwells for the Zero Standard and the samples to be worked. Place an equal number of Antibody Coated Microtiter Wells in another microwell holder. Immediately reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided. The samples should be stored in a cool place.

10.3 Add **100µl** of **1 Standard DON Detection Solution** to each Dilution Well.

10.4 Using new pipette tip for each, add **100µl** of **Standard 1** and prepared sample (see Chapter 9) to appropriate Dilution Well containing the **1 Standard DON Detection Solution**. Mix by priming pipetting at least 5 times.

10.5 Using a multichannel pipette, transfer **100µl** of contents from each Dilution Microwell to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for **2 minutes**.

10.6 Wash the plate as follows: Aspirate the liquid from each well into the sink and tap the holder of microwells upside down strongly (four times in a row) on an absorbent paper to insure the complete removal of liquid from the wells. Dispense **300µl** of **Wash Buffer 1X** (see 9.1) into each well with wash bottle or multichannel micropipette using the proper reagent reservoir and shaking the plate manually for a few seconds. Repeat this process for another two times **(total 3 times). CAUTION:** It is important to not allow microwells to dry between working steps.

10.7 Aspirate the liquid as described above and add **100µl** per well of **TMB Substrate** (pour 1ml per 8 wells in a reservoir). Shake the plate manually for a few seconds and incubate in the dark at room temperature for **3 minutes.**

10.8 Add **100µl** per well of the **Stop Solution** to each well (pour 1ml per 8 wells in a reservoir). Mix gently by shaking again the plate manually.

10.9 **Measure the absorbance at 450nm.** Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620nm as the reference wave length (610nm to 650nm is acceptable).

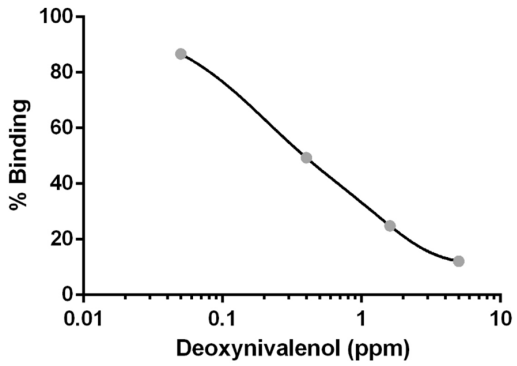
11. Data Analysis

- **Automatically**

An assigned software, the **Prognosis-Data-Reader,** is available for free (contact: info@prognosis-biotech.com) download in order to evaluate the 1 Standard DON ELISA kit. The evaluation is carried out by a simple transfer of data values after the measurement. B/Bo (%) values of the standards are reported in the Quality Assurance Certificate.

Alternatively, typing the lot number of the kit and the B/Bo (%) values can be automatically downloaded.

12. Example of Standard Curve (0 - 5ppm)



13. Performance Evaluation

13.1 Reference Materials

Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at info@prognosis-biotech.com.

14. Method Summary

Total procedure time (after samples and reagents preparation): 5min.

Mix 100µl of the Detection Solution with 100µl of Zero Standard and the samples in the Dilution Microwells

⇩

Transfer 100µl from each well of the Dilution Microwells into the Antibody Coated Microwells

⇩

Incubate 2min at room temperature

⇩

Wash three times

⇩

Add 100 µl of TMB Substrate

⇩

Let the color develop for 3min in the dark at room temperature

⇩

Add 100 µl Stop Solution

⇩

Read Absorbance at 450 nm within 60 min

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