

| VERSION 8

CAT.NUMBER: D4448

STORAGE: 2-8°C



STANDARD ZON

ELISA TEST | In vitro analysis

for the quantitative detection of Zearalenone in grains,
cereals and animal feed



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

www.prognosis-biotech.com

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 ZON, D4448, is an immunoassay method that determines the Zearalenone, 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 is included). A spectrophotometer for microtiter ELISA plate is required.

Matrices:

- **Cereals:** Barley, Biscuits, Bran sticks, Bread, Brown rice, Corn, Corn flakes, Corn flour, Cottonseed, DDGS, Oat bran, Oat flakes, Oats, Pasta, Pet food, Popcorn, Raw rye, Rice, Rye flour, Sesame, Wheat, Wheat bran, Wheat Flour

- Sample preparation: extraction
- Test time (incubation time after samples and reagents preparation): 5min
- Standard curve range: 0 - 500 ppb
- Shelf life: 12 months
- Storage: 2-8°C

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1. Description

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

2. General Information

Zearalenone (ZON) is a member of the trichothecene mycotoxins produced by fungi of the *Fusarium* genus (*F. graminearum*). Grains including barley, wheat, oats, corn, rice and maize are frequently infected by this fungus. It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperoestrogenic syndromes in humans. There is evidence that ZON and its metabolites possess oestrogenic activity in pigs, cattle and sheep. Moreover, ZON has also been shown to be hepatotoxic, haematotoxic, immunotoxic and genotoxic. Most controlling government agencies worldwide have regulations regarding the amount of ZON allowable in human and animal foodstuffs. Accurate and rapid determination of ZON 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 ZON specific antibodies. Toxins are extracted from a ground sample with methanol 70%. Zearalenone standards or samples and ZON-HRP conjugate (detection solution) are added into the coated wells. ZON-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by ZON of standards or samples. Any unbound ZON-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 ZON present in the samples and standards.

4. Reagents Provided

1 Standard ZON 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 (25 - 500 ppb) are reported in the Quality Assurance Certificate of each lot.

| Reagents (Store at 2-8°C) | 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 ppb of ZON in organic solution) | 1 glass vial (3.0 mL) | Ready to use | Black |
| 1 Standard ZON detection solution | 1 plastic vial (6 mL) | Ready to use | Green |
| Wash Buffer | 1 plastic vial (50 mL) | 20X Concentrate (dilute in distilled water) | White |
| TMB Substrate | 1 plastic vial (6 mL) | Ready to use | Brown |
| Stop Solution | 1 plastic vial (6 mL) | Ready to use | White |

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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 fitted with 450 nm filter
- 100, 200 and 1000 μ L adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100 μ l is acceptable for the steps of TMB and Stop Solution)
- 50 - 300 mL 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 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 (15% H₃PO₄) 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 (450 nm) for the Standard 1 (St1).

9. Sample and reagents preparation

9.1 Reagents Preparation

- Prepare the Extraction Solution (70% Methanol) by adding 30 mL of distilled or deionized water to 70 mL 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 (50 mL) into a clean 1000 mL graduated cylinder, rinse the vial with distilled or deionised water and pour the content again into the cylinder and fill to a final volume of 1000 mL with distilled or deionised 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.

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9.2 Sample Preparation

9.2.1 Ground Samples

- 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).
- 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.
- 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).**
- Use 100 μ L 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.

NOTE2: In case the user makes an additional dilution 1:1 of filtrate with methanol 14% the range of quantification becomes 0 - 1000 ppb. So, use also 100 μ L of each extra diluted filtrate directly in the immunoassay and multiply the final ZON ppb result x 2.

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 the **appropriate number of dilution wells** into the holder of microwells for the Zero Standard and the samples to be tested. 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 100 μ L of 1 Standard ZON Detection Solution to each Dilution Well.

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

10.5 Using a multichannel pipette, transfer **100 µL** 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 ensure the complete removal of liquid from the wells. Dispense **300 µL** 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 three times (**total 4 times**). **CAUTION:** It is important to not allow microwells to dry between working steps.

10.7 Aspirate the liquid as described above and add **100 µL** 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 **100 µL** of the **Stop Solution** to each well (pour 1ml per 8 wells in a reservoir). Mix gently by shaking again the plate manually.

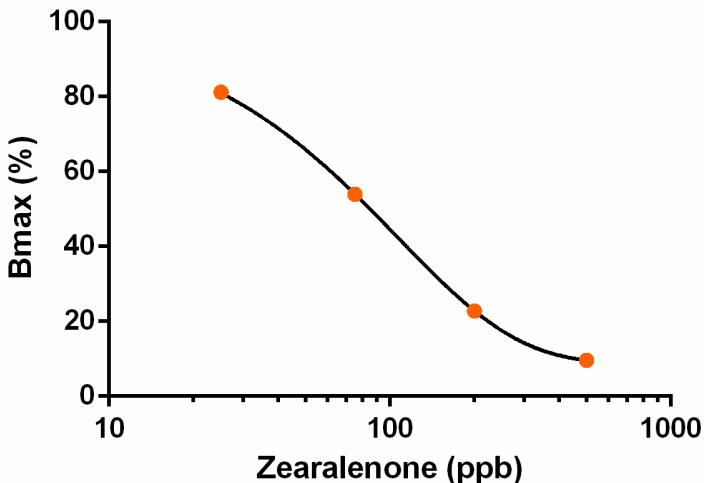
10.9 Measure the absorbance at 450 nm. Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620nm as the reference wave length (610 nm to 650 nm is acceptable).

11. Data Analysis

An assigned software, the **Prognosis-Data-Reader**, is available for free (contact: info@prognosis-biotech.com) download in order to evaluate the 1 Standard ZON 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 - 500 ppb)



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13. Immunoassay Specification

13.1 General Specification

- IC50 = 60 - 130 ppb

13.2 LOD - LOQ - Accuracy

- The LOD of the method is 20 ppb
- The LOQ of the method is 25 ppb
- The recovery of spiked extractions-matrices was 96.5% (CV = 6.8%)

- **Matrices:**

Cereals: Barley, Biscuits, Bran sticks, Bread, Brown rice, Corn, Corn flakes, Corn flour, Cottonseed, DDGS, Oat bran, Oat flakes, Oats, Pasta, Pet food, Popcorn, Raw rye, Rice, Rye flour, Sesame, Wheat, Wheat bran, Wheat Flour

13.3 Specificity

The cross-reaction of the anti-ZON antibody with Zearalenone, α -zearalenol, β -zearalenol, Zearalanone, α -zearalanol and β -zearalanol is 100, 80, 41, 93, 78 and 82% respectively.

14. Performance Evaluation

14.1 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.

15. Method Summary

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

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



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



Incubate 2 min at room temperature



Wash four times



Add 100 μ L of TMB Substrate



Incubate 3 min at room temperature



Add 100 μ L Stop Solution



Read Absorbance at 450 nm within 60 min

All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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