

| VERSION 8

CAT.NUMBER: D4748

STORAGE: 2-8°C



# STANDARD FUMONISIN

## **ELISA TEST | In vitro analysis**

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



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



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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 Fumonisin, D4748, is an immunoassay method that determines the Fumonisin, 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:** Alfalfa, Animal feed, Biscuits, Black Beans Flour, Borlotti Beans Flour, Bran sticks, Brown rice, Cannellini Beans Flour, Corn, Corn flakes, Corn flour, Cottonseed, Dried sunflower, Green Peas Flour, Nachos, Oat bran, Oat flakes, Oats, Pasta, Peas Flour, Pet food, Popcorn, Red Lentils Flour, Rice, Round Beans Flour, Sorghum, Soy beans, Soy flour, Wheat, Wheat bran, Yellow Chickpeas Flour, Yellow Lentils Flour
- **Other:** Cocoa, Milk

- Sample preparation: extraction
- Test time (incubation time after samples and reagents preparation): 5 min
- Standard curve range: 0 - 6 ppm
- Shelf life: 12 months
- Storage: 2-8°C

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## 1. Description

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

## 2. General Information

Fumonisin is a member of the trichothecene mycotoxins produced by fungi of *Fusarium moniliforme* (*F. verticillioides*), *F. proliferatum*, and several other *Fusarium* species. Grains including corn, wheat and other cereals are frequently infected by these fungi in the field or during storage. More than ten types of fumonisins have been isolated and characterized. Of these, fumonisin B1 (FB1), B2 (FB2), and B3 (FB3) are the major fumonisins produced. Fumonisin is hepatotoxic and nephrotoxic in all animal species tested. The earliest histological change to appear in either the liver or kidney of fumonisin-treated animals is increased apoptosis followed by regenerative cell proliferation, while the acute toxicity of fumonisin is low, it is the known cause of two diseases which occur in domestic animals with rapid onset: equine leukoencephalomalacia and porcine pulmonary oedema syndrome. Both of these diseases involve disturbed sphingolipid metabolism and cardiovascular dysfunction. Most controlling government agencies worldwide have regulations regarding the amount of FB1, and FB2 allowable in human and animal foodstuffs. Accurate and rapid determination of Fumonisin 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 Fumonisin specific antibodies. Toxins are extracted from a ground sample with methanol 70%. Fumonisin standards or samples and Fumonisin-HRP conjugate (detection solution) are added into the coated wells. Fumonisin-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by Fumonisin of standards or samples. Any unbound Fumonisin-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 Fumonisin present in the samples and standards.

## 4. Reagents Provided

1 Standard Fumonisin 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 (0.2 - 6 ppm) 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 ppm of Fumonisin in organic solution)	1 glass vial (3 mL)	Ready to use	Black
1 Standard Fumonisin 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

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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  $\mu$ L adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100  $\mu$ L is acceptable for the steps of TMB and Stop Solution)
- 50 - 300  $\mu$ L 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%  $H_3PO_4$ ) 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 (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 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 20g 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 100µl 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 make an additional dilution 1:1 of diluted filtrate with 14% methanol the range of quantification becomes 0 - 12 ppm. So, use also 100 µL of each extra diluted filtrate directly in the immunoassay and multiply the final Fumonisin ppm result x 2.

## 9.3 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 Fumonisin ppb result by 5. The LOD is 0.03ppm and the LOQ is 0.04ppm.

## 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) (Zero Standard included) 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 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.

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**10.3** Add **100  $\mu\text{L}$**  of **1 Standard Fumonisin Detection Solution** to each Dilution Well.

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

**10.5** Using a multichannel pipette, transfer **100  $\mu\text{L}$**  of contents from each Dilution Microwell to a corresponding Antibody Coated Microtiter Well. Cover the microwells with the sealing film and incubate at room temperature for **2 minutes**.

**10.6** Remove the sealing film and 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 **300  $\mu\text{L}$**  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  $\mu\text{L}$**  per well of **TMB Substrate** (pour 1ml per 8 wells in a reservoir). Cover the microwells with the sealing film, shaking the plate manually for a few seconds and incubate in the dark at room temperature for **3 minutes**.

**10.8** Remove the sealing film and add **100  $\mu\text{L}$**  of the **Stop Solution** to each well (pour 1ml per 8 wells in a reservoir). Mix gently by shaking again the plate manually.

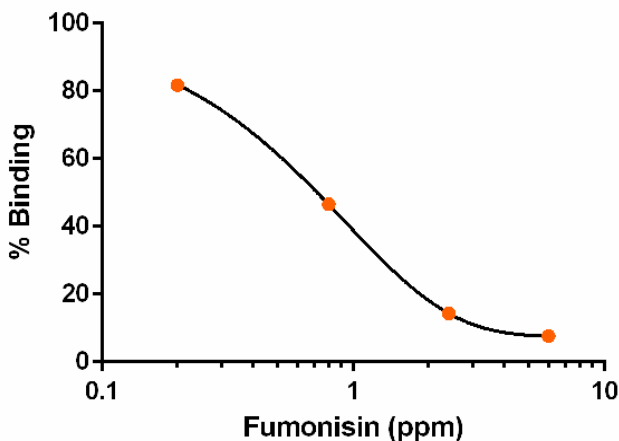
**10.9 Measure the absorbance at 450 nm.** Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

## 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 Fumonisin 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 - 6 ppm)



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## 13. Immunoassay Specification

### 13.1 General Specification

- IC50 = 0.4 - 1.2 ppm.

### 13.2 LOD - LOQ - Accuracy

- The LOD of the method is 0.15 ppm.
- The LOQ of the method is 0.20 ppm.
- The recovery of spiked extractions-matrices was 105% (CV = 8.3%).

#### • Matrices:

**Cereals:** Alfalfa, Animal feed, Biscuits, Black Beans Flour Precooked and Refined, Borlotti Beans Flour Precooked and Refined, Bran sticks, Brown rice, Cannellini Beans Flour Raw and Refined, Corn, Corn flakes, Corn flour, Cottonseed, Dried sunflower, Green Peas Flour Precooked and Refined, Nachos, Oat bran, Oat flakes, Oats, Pasta, Peas Flour Precooked, Pet food, Popcorn, Red Lentils Flour Raw and Refined, Red Lentils Flour Precooked and Refined, Rice, Round Beans Flour Precooked and Refined, Sorghum, Soy beans, Soy flour, Wheat, Wheat bran, Yellow Chickpeas Flour Precooked and Refined, Yellow Chickpeas Flour Raw and Refined, Yellow Lentils Flour Precooked and Refined, Yellow Lentils Flour Raw and Refined

**Other:** Cocoa, Milk

### 13.3 Specificity

The cross-reaction of the anti-Fumonisin antibody with FB1, FB2 and FB3 is 100, 65 and 42% 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](mailto:info@prognosis-biotech.com).

## 15. Method Summary

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

**Mix 100  $\mu$ L of the Detection Solution with 100  $\mu$ L of Zero Standard and the samples in the Dilution Microwells**



**Transfer 100  $\mu$ L from each well of the Dilution Microwells into the Antibody Coated Microwells**



**Incubate 2 min at room temperature**



**Wash four times**



**Add 100  $\mu$ L of TMB Substrate**



**Incubate 3 min at room temperature**



**Add 100  $\mu$ L Stop Solution**



**Read Absorbance at 450 nm within 60 min**



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