| VERSION 22

CAT.NUMBER: B2548/B2596

STORAGE: 2-8°C

BIO-SHIELD M1 FAST

ELISA TEST | In vitro analysis

for the quantitative detection of Aflatoxin M1 in milk and milk powder

PROGNOSIS BIOTECH

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

www.prognosis-biotech.com

This ELISA kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 14675: 2003

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.

Bio-Shield M1 FAST, B2548/B2596, is an immunoassay method that determines the Aflatoxin M1 in milk, milk powder, cheese and yogurt. The ELISA kit contains all reagents required for the immunoassay method. The ELISA test is adequate for 48/96 definitions (Zero Standard (St1) is included). A spectrophotometer for microtiter ELISA plate is required.

- Sample preparation: milk: no preparation, milk powder: reconstitution
- Test time: 30min
- Shelf life: 12 months
- Storage: 2-8^{o}C

1. Description

Bio-Shield M1 FAST is an ELISA test for the detection of Aflatoxin M1 in raw and homogenized milk and milk powder without sample preparation.

2. General Information

Aflatoxins are toxic metabolites of major concern to the dairy industry, generally produced by *Aspergillus fl avus, A. parasiticus* and *A. nomius.* They can have immunosuppressive, mutagenic, teratogenic and carcinogenic effects. Aflatoxins that are ingested by animals in contaminated pellets and forage are biotransformed at the hepatic level into Aflatoxin M1 (AFM1). Aflatoxin is then excreted in this form into the milk used for human consumption and, it is also present in dairy products. AFM1 in milk and milk products is considered to pose certain hygienic risks for human health and as a result there is an established EU limit 0.05 μg/kg (50 ppt).

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 AFM1 specific antibodies. AFM1 standards or samples are added into the wells of the microtiter plate. Then, AFM1 of standards or samples (if AFM is present) binds to the coated antibodies. Any unbound AFM1 is removed in a washing step. A detection solution with AFM1-HRP conjugate is added and it binds to the binding sites of coated antibodies that are not already occupied by AFM1 of standards or samples. Any unbound AFM1-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 AFM1 present in the samples and standards.

4. Reagents Provided

Bio-Shield M1 FAST ELISA kit contains sufficient reagents and materials for 96 measurements (including Zero Standard test). Zero Standard (St1) is the only standard provided and the B/Bo values of St2-St6 (10 - 250ppt) are reported in the Quality Assurance Certificate of each lot.

Reagents (Store at 2-8ºC)	Quantity for 48 wells	Quantity for 96 wells	State	Vial cap color
Single-Break Strip Plate	48 wells	96 wells	Ready to use (precoated)	-
Sealing film	2 sheets	2 sheets	Ready to use	-
Zero Standard (St1) (0 ppt of AFM1)	1 glass vial (3ml)	2 glass vials (each 3ml)	Ready to use	Black
M1 Detection Solution	1 plastic vial (6ml)	1 plastic vial (12ml)	Ready to use	Green
Wash Buffer	1 plastic vial (50ml)	1 plastic vial (50ml)	20X Concentrate (dilute in distilled water)	White
TMB Substrate	1 plastic vial (6ml)	1 plastic vial (12ml)	Ready to use	Brown
Stop Solution	1 plastic vial (6ml)	1 plastic vial (12ml)	Ready to use	White

5. Materials required but not provided

- Centrifuge, Magnetic stirrer, Vortex mixer and Microtiter plate reader fitted with 450 nm filter.
- 100 and 1000μl adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100μl is preferable for the steps of Detection Solution, TMB and Stop Solution).
- 50 - 300μl multi-channel micropipette with disposable tips and reservoirs.
- Distilled water

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, 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 standards (AFM1), 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 (ABS 450nm) for the Zero Standard (St1).

9. Sample and reagents preparation

9.1 Reagents preparation

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 deionised water and pour the content again into the cylinder and fill to a final volume of 1000ml 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.

9.2 Samples preparation

9.2.1 Milk

Use 100µl of each milk sample directly in the immunoassay. Centrifugation (3000xg for 10 min) is not neces-sary because there is no significant difference in the final result.

9.2.2 Milk Powder

Reconstitute the milk powder according to manufacturer's instructions. If there are no instructions available mix 1g of milk powder deionized or distilled water until 10ml. Mix well and afterwards there follows the skim-ming according to the sample preparation of milk (see 9.1). Use 100µl of each sample directly in the immuno-assay.

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 standards. 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 48 wells (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 wells** into the holder of microwells for the Zero Standard and the samples to be worked. Place the wells into the holder of microwells and immediately reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.

10.3 Milk can be used directly in the assay with no preparation. The samples should be stored in a cool place. Add **100 µl** per well of Zero Standard (**St1**) or sample (see Chapter 9 in case of powder). Cover the microwells with the sealing film, shake the microplate manually for 30 seconds and incubate at room temperature for **20 minutes**.

10.4 Remove the sealing film and wash the plate as follows: Aspirate the liquid from each well (100µl/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 three times (**total 4 times**). **CAUTION**: It is important to not allow microwells to dry between working steps.

10.5 Aspirate the liquid as described above and add **100µl** of **Detection Solution** to each well. If the number of wells is more than 32 (four strips), a repetitive pipette or multichannel pipette is necessary (pour 1 ml of Detection Solution in a reservoir per 8 wells). Cover the microwells with the sealing film, shake the plate manually for a 30 seconds and incubate at room temperature for **5 minutes .**

10.6 Remove the sealing film and wash the plate as the wash **step 10.4.**

10.7 Aspirate the liquid from each well and tap the holder of microwells upside down strongly on the absorbent paper as described above and add **100 µl** 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 **5 minutes**.

10.8 Remove the sealing film and 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.

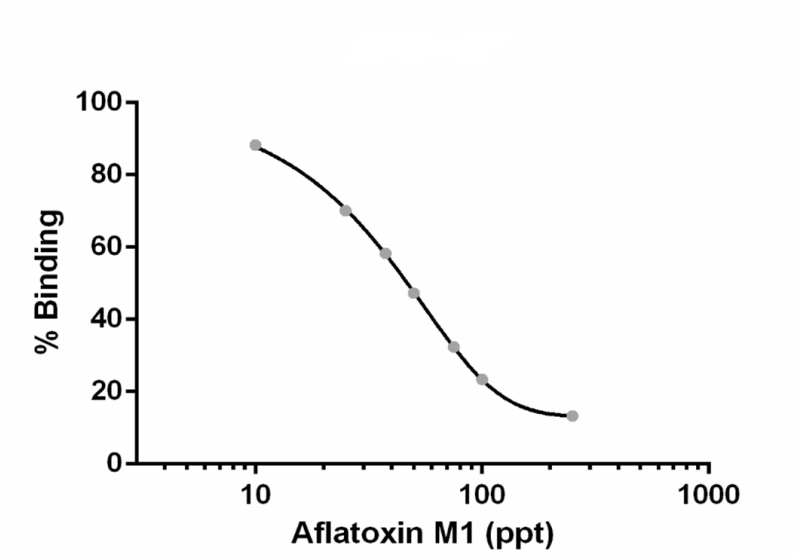
10.9 **Measure the absorbance at 450nm.** Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

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 Bio-Shield M1 FAST 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 - 250ppt)



13. Immunoassay Specification

13.1 General Specification

- Coefficient of Variation (CV) of result at 50ppt = 8.25% (n=16)

13.2 LOD - LOQ - Recovery

		Raw and homogenized milk	Whole milk powder
LOD		5ppt	5ppt
LOQ		10ppt	10ppt
Accuracy (of result)	Recovery (concentrations between 25 and 100ppt of AFM1)	100% ± 20%	100% ± 20%

13.3 Specificity

The cross-reaction of the anti-Aflatoxin M1 antibody with Aflatoxin M1 and Aflatoxin M2 is 100 and <0.1% 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.

14. Method Summary

Total procedure time (after samples and reagents preparation): 30 min.

Add 100 µl of each standard and sample in microplate

⇩

Incubate 20min at room temperature

⇩

Wash four times

⇩

Add 100 µl of Detection Solution

⇩

Incubate 5min at room temperature

⇩

Wash four times

⇩

Add 100 µl of TMB Substrate

⇩

Let the color develop for 5min in the dark at room temperature

⇩

Add 100 µl 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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www.prognosis-biotech.com
e: info@prognosis-biotech.com
t: +30 2410 623922 | f: +30 700 700 6262
Farsalon 153 | 41335 Larissa, Greece