VERSION N2
CAT.NUMBER: B2248/B2296

# BIO-SHIELD M1 UF MYD

**ELISA TEST | In vitro analysis**

for the quantitative detection of Aflatoxin M1 in milk, milk powder and yogurt drink

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 ULTRA FAST for Milk and Yogurt Drink, B2248/B2296, is an immunoassay method that determines the Aflatoxin M1 in milk, milk powder and yogurt drink. The ELISA kit contains all reagents required for the immunoassay method. The ELISA test is adequate for 48/96 definitions (standards are included). A spectrophotometer for microtiter ELISA plate is required.

Sample preparation:

- milk: no preparation
- milk powder: reconstitution
- yogurt drink: dilution

Test time : 20min

Shelf life: 12 months

Standard curve range for milk: 0 - 1000ppt

Standard curve range for yogurt drink: 0 - 1500ppt

Storage: 2-8$^{o}$C

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

**Specifications**

- IC50 = 150 - 450 ppt
- Each standards duplicates mean CV ≤ 6%
- Coefficient of Variation (CV) of result at 500ppt = 5.85% (n=16)
- LOD - LOQ - Recovery

| | | Raw and homogenized milk | Whole milk powder | Yogurt Drink |
|---|---|---|---|---|
| **LOD** | | 20ppt | 20ppt | 30ppt |
| **LOQ** | | 70ppt | 70ppt | 105ppt |
| **Accuracy (of result)** | Recovery (concentrations between 250 and 1000ppt of AFM1) | 99.4% | 110% | 106% |

- The cross-reaction of the anti-Aflatoxin M1 antibody with AFM1and AFM2 is 100 and <0.1% respectively.

**1. Description**

Bio-Shield M1 ULTRA FAST for milk and yogurt drink is an ELISA test for the detection of Aflatoxin M1 in raw and homogenized milk, milk powder and yogurt drink.

**2. General Information**

Aflatoxins are toxic metabolites of major concern to the dairy industry, generally produced by *Aspergillus flavus, 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 American and Asian limit 0.5 µg/kg (500 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 and AFM1-HRP conjugate (detection solution) are added into the wells of the microtiter plate. Then, AFM1-HRP conjugate 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 Ultra Fast for milk and yogurt drink ELISA kit contains sufficient reagents and materials for 48/96 measurements (including standard tests).

| 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) | - |
| Dilution Microwells | 48 wells | 96 wells | Ready to use (red color) | - |
| Sealing film | 2 sheets | 2 sheets | Ready to use | - |
| Matrix Diluent | 1 plastic vial (12ml) | 2 plastic vials (12ml) | Ready to use | Red |
| Standards 1-5 (0, 100, 250, 500 and 1000ppt of Aflatoxin M1) | 5 plastic vials (each 1.5ml) | 5 plastic vials (each 1.5ml) | Ready to use | Brown |
| 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 |
| Yogurt Drink Buffer | 1 plastic vial (50ml) | 1 plastic vial (50 ml) | 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 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 standards (AFM1), 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 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 50µl of each milk sample directly in the immunoassay. Centrifugation (3000xg for 10 min) is not necessary 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 skimming according to the sample preparation of milk (see 9.1). Use 50µl of each sample directly in the immuno-assay.

***9.2.3 Yogurt Drink***

In a 2ml tube add 1mL (or 1g) of yogurt drink sample and 0.5mL of Yogurt Drink Buffer. Mix well by vortex (15 sec). Use 50µl of each mixture directly in the immunoassay. The dilution factor is 1.5. So, multiply the final Aflatoxin M1 ppt result x 1.5.

**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 should be tested in duplicate, create a layout.

**CAUTION:** Use the standards positions in duplicate as the **Example plate** layout below **NECESSARY** and note positions of samples that can be set to all remaining empty wells of layout in duplicate.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | St1 | St1 | | | | | | | | | | |
| B | St2 | St2 | | | | | | | | | | |
| C | St3 | St3 | | | | | | | | | | |
| D | St4 | St4 | | | | | | | | | | |
| E | St5 | St5 | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

**Example plate layout (example for a 5 point standard)**

***10.2*** Bring all reagents to room temperature (19 - 24°C) before use. Remove the **standards** (Standard 1-5) and place two Dilution Microwells (red) in a microwell holder for each Standard and Sample to be tested in duplicate. 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 **200µl** of Matrix Diluent to each Dilution Well.

***10.4*** Using new pipette tip for each, add **50µl** of each Standard **(Standard 1 - 5)** and prepared sample in duplicate (see Chapter 9) to appropriate Dilution Well containing the Matrix Diluent. 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. Cover the microwells with the sealing film and incubate at room temperature for **10 minutes**.

***10.6*** Remove the sealing film and wash the plate as follows: Aspirate the liquid from each well (200µl/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.

***10.7*** Aspirate the liquid as described above and add **100µl** of **Detection Solution** to each well using a multichannel pipette, (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.**

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

***10.9*** 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**.

***10.10*** 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.

***10.11*** **Measure the absorbance at 450nm.** Read the absorbance value of each well (within 60 minutes after the step 10.10) 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

- **Automatically**

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 Ultra Fast for milk and yogurt ELISA kit. The evaluation is carried out by a simple transfer of data values after the measurement.

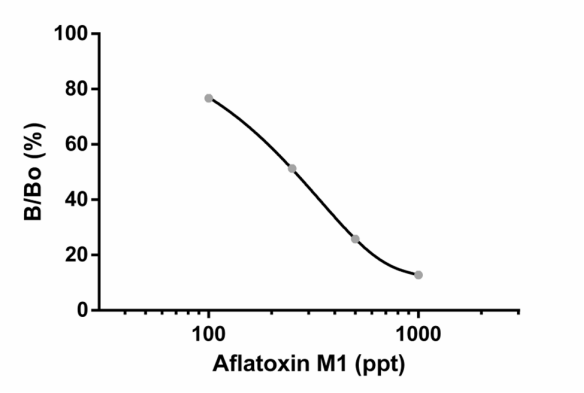
- **Manually**

Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 10% of the mean. Use the following calculation:

$$\frac{\text{Standard or sample absorbance}}{\text{Standard 1 absorbance}} \times 100 = \%\ \text{Binding}$$

The standard 1 is equal to 100 % and the absorbance values are quoted in percentages. The concentration of Aflatoxin M1 (ppt) in each sample is determined by extrapolating OD values against concentrations of Aflatoxin M1 in standard solutions using a two phase exponential decay standard curve with logarithmic X axis.

## 12. Example of Standard Curve (0 - 1000ppt)




## 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): 20 min.

**Mix 200µl of the Matrix Diluent with 50µl of the samples and standards in the Dilution Microwells**

⇩

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

⇩

**Incubate 10 min 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**



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