BZERO AFLA M_1 500

Enzyme immunoassay for the detection of Aflatoxin M_1 (Cat.nr.HU0040091)

BZERO AFLA M₁ is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of aflatoxin M_1.
The kit contains the procedure and the materials sufficient for 96 determinations, zero standard included.
For result evaluation a microtiter plate or a strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Raw milk, milk powder.

Sample preparation
- Raw milk: no preparation required.
- Milk powder: dilution.

Assay time: 20 minutes (sample preparation not included).

Detection limit
- Raw milk: 25 ng/l
- Milk powder: 250 ng/l

Specificity	
Analyte	**Cross-reactivity (%)**
Aflatoxin M_1	100

1. TEST PRINCIPLE

The assay is performed in plastic microwells which have been coated with anti-Aflatoxin M_1 antibodies. Aflatoxin M_1 standard solutions and samples are added to the microwells.
During the first incubation, free Aflatoxin M_1 molecules are bound to the anti-Aflatoxin M_1 antibodies.
Any unbound substance is then removed in a washing step.
A second incubation is performed with an aflatoxin-HRP conjugate, which covers all the remaining free binding sites of the antibody. After the incubation a second washing step is performed.
The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colorless chromogen into a blue product during the third incubation.
The addition of the stop reagent leads to a color change from blue to yellow. The absorbance is measured by a microplate reader at 450_{nm}. The color development is inversely proportional to the Aflatoxin M_1 concentration in the sample.

2. PROVIDED REAGENTS

Microtiter plate: 96 wells (12 strips x 8 wells) coated with anti-Aflatoxin M_1 antibodies.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the frame and to break the joint.
Std 0: 1 plastic vial containing 3 ml of 0 ng/l of aflatoxin M_1.
ATTENTION: Only the zero standard is provided. B/B_0 values of calibration curve (25-500 ng/l) are reported in the kit certificate of analysis.
Enzyme conjugate: 1 plastic bottle containing 14 ml.
Washing-buffer 20X: 1 plastic bottle containing 50 ml.
Developing solution: 1 plastic bottle containing 14 ml.
Stop solution: 1 glass vial containing 8 ml. White cap.

3. MATERIALS REQUIRED BUT NOT PROVIDED

- *"Milk Diluent" (cat.nr.HU0040101)*
- Distilled water

Equipment
- Balance
- Micropipette 50-200 µl, tips
- Multichannel micropipette 50-250 µl, if using more than three strips
- Microplate reader, filter 450nm

4. WARNING AND PRECAUTIONS FOR THE USERS

- The test for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) N° 1272/2008.

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Please refer to *Material Safety Data Sheet* available on both the Eurofins Technologies and Eurofins Tecna *(tecna.eurofins-technologies.com)* web site.

- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (2 hours). ATTENTION:** Do not unseal the microplate until it reaches the room temperature.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components between different kit lots.
- Do not use photocopies of the instruction booklet. Keep always to the instruction booklet which is included inside the kit
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times;
  - do not incubate the plate at temperatures higher than 25°C or lower than 18°C;
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- Use a single disposable tip for each sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells or the internal microwells surface.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.

6. SAMPLES PREPARATION

6.1 Raw milk

After milking, the milk has to be tested within 24 hours. Otherwise milk has to be stabilized (with sodium azide, azidiol or similar substances)

1) Adjust the milk to room temperature.
2) Use the milk directly in the assay, without skimming.
3) If the sample is dosed >500 ng/l, it is suggested to dilute it four times (1+3) in “Milk Diluent” (cat.nr. HU0040101) and retest it, in order to obtain a dosage range 100-2000 ng/l.

6.2 Powdered milk

1) Weight out 10 g of the powder and get to a volume of 100 ml with distilled water (it is suggested to heat the water to 37°/40 °C to dissolve the powder easier).
2) Shake until the powder is completely dissolved.
3) The dilution factor is 10.

7. WORKING SOLUTIONS PREPARATION

0 Std: ready to use; mix before use.
Enzyme conjugate: ready to use.
Washing buffer: dilute the concentrate 1:20 (1+19) with distilled water; **ATTENTION:** In presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light;
Stop solution: ready to use. **ATTENTION:** It contains 1 M sulphuric acid. Handle with care; in case of contact flush immediately with plenty of water.

8. ASSAY PROCEDURE

1) Predispose the assay layout, recording standard and samples positions, taking into account that one well is required for each sample and standard 0.
   ATTENTION: It is suggested to carry out no more than 24 determinations in each assay (standard 0 included).
2) First incubation
   - Add 100 µl of standard 0/ sample into the corresponding wells.
   - Shake the plate gently with rotatory motion for few seconds
   - Incubate 10 minutes at room temperature.
   - Do not prolong the first incubation time and do not use automatic shakers.
3) Washing
   - Pour the liquid out from the wells.
   - Fill completely all the wells with working wash solution using a squeeze bottle. Pour the liquid out from the wells.

- Repeat the washing sequence three (3) times.
- Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out.

4) Second incubation
   - Using a multichannel pipet, add to the wells 100 µl of the enzyme conjugate solution.
   - Shake the plate gently with rotatory motion for few seconds.
   - Incubate for 5 minutes.
5) Repeat step 3.
6) Developing
   - Using the multichannel pipet, add 100 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 5 minutes at room temperature.
7) Using a multichannel pipet, add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
8) Measure the absorbance at 450 nm.
9) Read within 15 minutes.

9. RESULTS CALCULATION

- Divide the absorbance value of each sample by the absorbance of the standard 0 (B0) and multiply by 100; the standard 0 (B0) is thus made equal to 100% and all the other absorbance values are expressed as percentage:

$$\frac{\text{Standard (or sample) absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0}\ (\%)$$

- Enter the B/B_0 values provided for each standard (25; 50; 200; 500 ng/l) in the kit lot analysis certificate in a semi-logarithmic system of coordinates against the aflatoxin M_1 standard concentration and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration from the calibration curve.
- The concentration of aflatoxin M_1 in the sample is to be calculated by multiplying the concentration read on the curve for the dilution factor.

Please note: for results calculation it is suggest to use the "point to point" curve. Excel spreadsheet are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 11).
If the values are outside the specifications given, then the results of the test are not assured, therefore the aflatoxin M_1 concentration levels in the samples may not be valid.
In these cases it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.

WARNING: Kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

11. KIT SPECIFICATIONS

11.1 Assay specification

Description	Specifications
B_0 absorbance	$\geq$ 0.7 OD_{450nm}

12. LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.

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