Importance of Aflatoxin M₁ Determination

Aflatoxins are highly toxic mycotoxins produced by a variety of molds such as Aspergillus flavus, A. parasiticus and A. nomius. These toxins are known carcinogens and can be present in grains, nuts, cottonseed and other foods consumed by humans or in animal feed.

Crops may be contaminated by some of these toxins during growth, harvest or storage. The toxins most frequently detected are Aflatoxin B₁, B₂, G₁, and G₂. When animals are fed contaminated feed, Aflatoxin B₁ is converted to M₁ by hydroxylation, which is subsequently secreted into the milk of lactating animals. Human breast milk can also contain Aflatoxin M₁ if a lactating woman has consumed food contaminated with Aflatoxin B₁.

Aflatoxin M₁ is very stable throughout milk processing methods such as pasteurization.

To protect humans, regulatory agencies around the world have imposed regulatory limits regarding the amount of Aflatoxins that are allowable in human and animal foods. In Europe, the Aflatoxin M₁ tolerance levels (ML) are as follows:

- Milk (raw milk, milk used in the production of milk based products and heat treated milk): 0.05 ppb
- Infant formula and infant milk: 0.025 ppb
- Dietary foods intended for infants: 0.025 ppb

The Aflatoxin M₁ ELISA allows the determination of 42 samples in duplicate determination. Less than 1 mL of sample extract is required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The detection limit for Aflatoxin M₁ is 11 pg/mL (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B₀) is at approximately 100 pg/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Specificity: The cross-reactivity of the Abraxis Aflatoxin M₁ Kit for various other Aflatoxins (B₁, B₂, G₁, and G₂) in milk were not determined as these mycotoxins are not excreted in milk.

Recoveries: Recoveries of Aflatoxin M₁ from spiked whole milk are as follows:

<table>
<thead>
<tr>
<th>M₁ Spiked (ppt)</th>
<th>M₁ Recovered (ppt)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30.3</td>
<td>100.6</td>
</tr>
<tr>
<td>100</td>
<td>104.6</td>
<td>104.6</td>
</tr>
<tr>
<td>200</td>
<td>188.1</td>
<td>94.1</td>
</tr>
<tr>
<td>Average Recovery</td>
<td></td>
<td>99.8</td>
</tr>
</tbody>
</table>

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India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India
Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444
Email: customerservice@lifetechnindia.com, www.atzlabs.com ; www.lifetechnindia.com

Aflatoxin M₁ ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Aflatoxin M₁ in Contaminated Samples

Product No. 53012M

1. General Description

The Aflatoxin M₁ ELISA is an immunoassay for the quantitative and sensitive screening of Aflatoxin M₁. This test is suitable for the quantitative and/or qualitative screening of Aflatoxin M₁ in milk and milk products (please refer to the appropriate technical bulletins for extraction/dilution procedures). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions of the test kit contain small amounts of Aflatoxin M₁. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Aflatoxin M₁ ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a forward ELISA and it is based on the recognition of Aflatoxin M₁ by antibodies. The calibrators and sample extract(s) are pipetted into test wells coated with Aflatoxin M₁ antibody to initiate the reaction. After a 30 minute incubation and washing of the wells, Aflatoxin-M₁ HRP conjugate is added, followed by a 60 minute incubation period. The HRP conjugate binds to unbound sites on the Aflatoxin M₁ antibody. Following this 60 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Aflatoxin M₁ HRP conjugate. After washing with the diluted wash solution, a clear substrate is added to the wells and any bound enzyme conjugate causes the conversion of the colorless substrate to a blue color. Following a 20 minute incubation, the reaction is stopped and the amount of color in each well is read using an ELISA reader. The color of the unknown samples is compared to the color of the calibrators and the Aflatoxin M₁ concentration of the samples is derived. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Aflatoxin M₁ ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can’t be completely excluded. Milk fats will cause interference in the test, therefore milk samples should be defatted as instructed in the sample preparation step (Section H) before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors can be: inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures (lower than 10 °C or higher than 30 °C) during the test performance.
The Abraxis Aflatoxin M1 ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.
**A. Reagents and Materials Provided**

1. Microtiter plate (12 X 8 strips) coated with polyclonal anti-Aflatoxin M1 antibody, in a resealable aluminum pouch
2. Calibrators/Standards (5): 0, 15, 25, 50, 100, 250 pg/mL (ppt) of Aflatoxin M1, 1 mL each
3. Aflatoxin M1-HRP Conjugate, 12 mL
4. Wash Solution (5X Concentrate), 100 mL. Must be diluted 1:5 with deionized water before use
5. Substrate (Color) Solution (TMB), 12 mL
6. Stop Solution, 6 mL (Handle with care)
7. Microtiter plate washer (optional)
8. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
9. Microtiter plate reader (wave length 450 nm)
10. Deionized or distilled water
11. Substrate (Color) Solution (TMB), 12 mL
12. Stop Solution, 6 mL (Handle with care)

**B. Assay Procedure**

1. Add 100 µL of the sample extracts (Section H) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Incubate for 30 minutes at room temperature.
3. Wash the strips three times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
4. Incubate the strips for 60 min at room temperature.
5. Wash the strips three times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of substrate (color) solution to the wells. Incubate the strips for 20 min at room temperature. Protect the strips from direct sunlight.
7. Add 50 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

**C. Evaluation**

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as Logit/Log or 4-Parameter (preferred). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Zero Standard (standard 0) and subtracting the result from 100.

**D. Working Scheme**

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

**E. Additional Materials**

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Calibrators/Standards (5): 0, 15, 25, 50, 100, 250 pg/mL (ppt) of Aflatoxin M1, 1 mL each
3. Aflatoxin M1-HRP Conjugate, 12 mL
4. Wash Solution (5X Concentrate), 100 mL. Must be diluted 1:5 with deionized water before use
5. Substrate (Color) Solution (TMB), 12 mL
6. Stop Solution, 6 mL (Handle with care)
7. Microtiter plate reader (wave length 450 nm)
8. Deionized or distilled water
9. Microcentrifuge capable of spinning at 12,000 – 14,000 RPM
10. Microcentrifuge tubes

**F. Working Scheme**

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.