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# Product Information

**Total Aflatoxin ELISA Kit Low Matrix (Qual.)**
for grains, cereal, silage, nuts, spices, and animal feed

Catalog Number **SE120008**
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Aflatoxins are toxic metabolites that different molds like *Aspergillus flavus* and *Aspergillus parasiticus* produce. Aflatoxins are carcinogenic and can be present as contaminants in grains, nuts, cottonseed, and other materials, e.g. crops, associated with animal feed or human food. In particular, four aflatoxin sub-types, $B_1$, $B_2$, $G_1$, and $G_2$ are known to occur as crop contaminants.[1,2]

The Total Aflatoxin ELISA Kit Low Matrix (Qual.) is a solid-phase direct competitive enzyme immunoassay. An aflatoxin-specific antibody which has been optimized to cross react with all four subtypes of aflatoxin ($B_1$, $B_2$, $G_1$, and $G_2$) is coated to a polystyrene microwell. Toxins are extracted from a ground sample with either 80% methanol or 80% acetonitrile, and, after dilution, added to the appropriate well. If aflatoxin is present, it will bind to the coated antibody. Subsequently, aflatoxin bound to horseradish peroxidase (HRP) is added and binds to the antibody not already occupied by aflatoxin present in the sample or standard. After this incubation period, the contents of the wells are decanted and washed. An HRP substrate is added, which develops a blue color in the presence of enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin in the standard or sample. Therefore, as the concentration of aflatoxin in the sample or standard increases, the intensity of the blue color will decrease. A solution containing a red dye is added to stop the enzyme reaction and simultaneously causes a color change, which leaves the bluest wells deep blue/purple and the lightest wells a mauve/pink. This facilitates a visual qualitative estimate of aflatoxin in the sample by comparing to the color of the standards.

This assay is intended for the qualtitative detection of aflatoxin $B_1$, $B_2$, $G_1$, and $G_2$ in grains, nuts, cottonseeds, cereals, and other commodities which are difficult to measure due to high matrix effects, such as silage and most spices.

### Components

1. Aflatoxin Low Matrix Microplate (981AFL01LM-QL): 96 wells (12 × 8 well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody
2. Aflatoxin Low Matrix Standards (983S1AFL01LM-QL, Black Cap): 3 vials, 1.5 mL/vial of aflatoxin at the following concentrations: 0.0, 0.1, 0.2 ng/mL in 50% methanol
3. Aflatoxin Low Matrix HRP-Conjugate (984MAFL01LM-QL, Green Cap): 12 mL of aflatoxin $B_1$ conjugated to peroxidase in buffer with preservative
4. Assay Diluent (937AD001, Brown Cap): 2 × 12 mL of proprietary sample diluent
5. TMB Substrate (916T001, Blue Cap): 12 mL of stabilized 3,3′,5,5′-tetramethylbenzidine (TMB)
6. Red Stop Solution (946PR001, Red Cap): 12 mL of Acidic Solution
7. PBST Wash Buffer Powder (915X001): 1 packet of PBS with 0.05% TWEEN® 20. Bring to 1 liter with distilled water and store refrigerated.
8. Mixing Wells (Red): 96 non-coated wells (12 × 8 well strips) in a microwell holde.

### Reagents and Equipment Required but Not Provided.

1. Grinder sufficient to render sample to particle size of fine instant coffee
2. Microplate reader capable of measuring absorbance at 650 nm
3. Precision pipettes to deliver 100–200 µL volumes
4. Collection Container: Minimum 250 mL capacity
5. Graduated cylinder: 100 mL
6. Methanol or acetonitrile: sufficient quantities for 80 mL reagent grade per sample
7. Distilled or deionized water: sufficient quantities for 20-50 mL per sample
8. Filter Paper: Whatman® #1 or equivalent
9. Filter funnel
10. Absorbent paper towels
11. Graph paper or computer and software for ELISA data analysis

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin. Wear protective gloves and safety glasses when using this kit.

**Storage/Stability**
Store reagents at 2–8 °C, and do not use beyond expiration date(s). Never freeze the kit components.

HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

**Procedures**
Notes:
Bring all reagents to room temperature (19–27 °C) before use. Do not return unused reagents back to their original bottles.

Before doing the assay, prepare a waste container as a receptacle for kit waste. Eject contaminated pipette tips and all other related materials into this container. Following completion of the assay, treat the container with sufficient 5-6% sodium hypochlorite (NaOCl) to saturate the container's contents, about $1/10^{th}$ the volume of the container. 5-6% NaOCl will denature the mycotoxins and neutralize the waste, which renders the waste safe for disposal. Invert the container several times to coat all waste thoroughly.

(In case of an accidental toxin spill, treat the spill surface with 5-6% NaOCl for a minimum of 10 minutes, and then with 5% aqueous acetone. Wipe dry with absorbent paper towels.)

Extraction Procedures / Sample Preparation
Note: Samples must be collected according to established sampling techniques.
*Corn, Wheat, Hay, Snaplage, Paprika, and Pistachio*
1. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Prepare Extraction Solvent (80% methanol or 80% acetonitrile) by adding 20 mL of distilled or deionized water to 80 mL of methanol or acetonitrile, for each sample to be tested.
3. Transfer 100 mL of the Extraction Solvent to a container and add 20 g of the ground sample. Note: The ratio of sample to Extraction Solvent is 1:5 (w/v).
4. Mix by shaking in a sealed container or in a blender for a minimum of 2 minutes.
5. Allow the particulate matter to settle. Then filter 5–10 mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. Alternatively, centrifuge the sample at 3,500 rpm for 5 minutes to pellet the particulate matter, and collect the supernatant for analysis.
6. Dilute an aliquot of the extract 5-fold with reconstituted PBST wash buffer.
7. The sample is now ready. The standards require no pre-dilution before use.
8. Final dilution for use in calculation = 25-fold

*Animal Feed*
1. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Prepare Extraction Solvent (80% acetonitrile) by adding 20 mL of distilled or deionized water to 80 mL of acetonitrile, for each sample to be tested.
3. Transfer 200 mL of the Extraction Solvent to a container and add 2 g of the ground sample. Note: The ratio of sample to Extraction Solvent is 1:100 (w/v).
4. Mix by shaking in a sealed container or in a blender for a minimum of 10 minutes.
5. Allow the particulate matter to settle. Then filter 5–10 mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. Alternatively, centrifuge the sample at 3,500 rpm for 5 minutes to pellet the particulate matter, and collect the supernatant for analysis.
6. Dilute an aliquot of the extract 5-fold with reconstituted PBST wash buffer.
7. The sample is now ready. The standards require no pre-dilution before use.
8. Final dilution for use in calculation = 500-fold

*Potable water*
This assay may be used for detecting aflatoxins in potable water. For these samples, 100 µL of the potable water sample should be used directly, as is, in the Assay Procedure without pre-dilution with the PBST wash buffer.

Assay Procedure

1. Reconstitute the PBST buffer into 1 L of distilled water. The remaining content packets may be washed out with a gentle stream of distilled water. Store the reconstituted buffer refrigerated, when not in use.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody-Coated Microwells in another microwell holder.
3. Dispense 200 μL of the Sample Diluent into each mixing well.
4. Using a new pipette tip for each, add 100 μL of each Standard and prepared Sample to the appropriate Dilution Well which contains diluent. Mix by priming pipettor at least 3 times.
   Note: The location of each Standard and Sample must be recorded throughout the test.
5. Using a new pipette tip for each, transfer 100 μL of contents from each Dilution Well to a corresponding Antibody-Coated Microwell. Incubate at room temperature for 30 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBST wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual PBST wash buffer.
8. Add 100 μL of Aflatoxin HRP-conjugate to each antibody-coated well and incubate at room temperature for 30 minutes. Cover to avoid direct light.
9. Repeat Steps 6 and 7.
10. Measure the required volume of Substrate Solution (1 mL/strip or 120 μL/well) and place in a separate container. Add 100 μL to each microwell. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
11. Measure the required volume of Stop Solution (1 mL/strip or 120 μL/well) and place in a separate container. Add 100 μL in the same sequence and at the same pace as the Substrate Solution was added.
12. Compare the color of the sample wells to the standards with the appropriate dilution factor to determine compliance with local and internationally accepted limits for aflatoxin. Alternatively, a permanent record can be made by reading the OD of the wells at 650 nm.

**Results**

Interpretation

The information contained on the label of each standard vial refers to the contents of that vial. However, the sample has been diluted at a 5:1 ratio by extraction solvent, as instructed in the Extraction Procedure. The sample is further diluted 5-fold with wash buffer (except animal feed and potable water). Thus the level of aflatoxin shown by the standard must be multiplied by 25 to indicate the ng per gram (ppb) of the commodity.

| Standard ng/mL | Commodity (ppb) |
|---|---|
| 0.0 | 0.0 |
| 0.1 | 2.5 |
| 0.2 | 5.0 |

For animal feed, the dilution factor is 500. The value of the standard must be multiplied by 500 to indicate the ng per gram (ppb) of the feed.

In the case of potable water, there is no pre-dilution. Thus the aftlatoxin is measured with a sensitivity equal to the lowest standard, which is 100 parts per trillion (ppt).

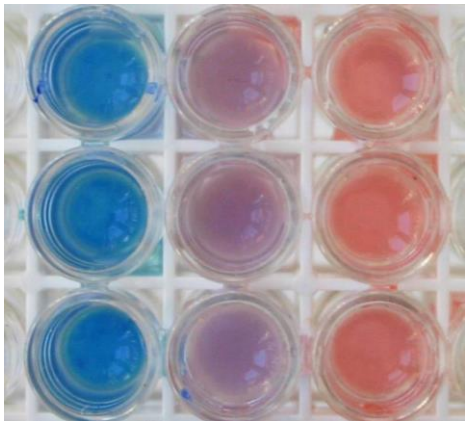
Assay Characteristics

The assay is intended primarily to give a qualitative/ semi-quantitative result in the following ranges, for particular sample types, as illustrated in Figure 1:

- Corn, Wheat, Hay, Snaplage, Paprika, and Pistachio (* in Figure 1): 0–5 ppb
- Animal Feed (** in Figure 1): 0–100 ppb

Values within each of these ranges for the respective samples may be easily distinguished by visual inspection. Aflatoxin values above these ranges for the respective samples will not be readily distinguished by visual inspection.

**Figure 1:**
Aflatoxin concentration in ppb, from sample data using corn samples or animal feed samples



| | | | |
|---|---|---|---|
| 0 | 2.5 | 5.0 | (*) |
| 0 | 50.0 | 100.00 | (**) |

(*) Corn samples were spiked with aflatoxin at 0, 2.5, and 5.0 ppb, and subsequently extracted and diluted 25-fold, to give the respective color samples.

(**) Animal feed samples were spiked with aflatoxin at 0, 50, and 100 ppb, and subsequently extracted and diluted 500-fold, to give the respective color samples.

- Corn, Wheat, Hay, Snaplage, Paprika, and Pistachio (* in Figure 1): 0–5 ppb
- Animal Feed (** in Figure 1): 0–100 ppb

Specificity
Cross Reactivity:
The assay cross-reacts with aflatoxin analogues as follows: $B_1$: 100%, $B_2$: 77%, $G_1$: 64%, $G_2$: 25%.

**References**

1. Klich, M.A., Environmental and developmental factors influencing by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience,* **48(2)**, 71-80 (2007).
2. Williams, J.H., *et al.*, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr.*, **80(5)**, 1106-1122 (2004).

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