

## Product Information

### Aflatoxin B<sub>1</sub> Low Matrix ELISA Kit

for grains, cereal, silage, nuts, spices, and animal feed

Catalog Number **SE120002**

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. Aflatoxin B<sub>1</sub> is the most toxic and frequently detected aflatoxin.<sup>1-3</sup>

The Aflatoxin B<sub>1</sub> Low Matrix ELISA kit is a solid-phase competitive enzyme immunoassay. An aflatoxin B<sub>1</sub>-specific antibody is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 50% methanol, 80% methanol, or 80% acetonitrile and after dilution, added to the appropriate well. If aflatoxin B<sub>1</sub> is present, it will bind to the coated antibody.

Subsequently, aflatoxin B<sub>1</sub> 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 analyzed using an HRP substrate, which develops a blue color in the presence of HRP. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin B<sub>1</sub> 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. An acidic stop solution is added, which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD<sub>450</sub>). The optical densities of the samples are compared to the ODs of the kit standards, and a result is determined by interpolation from the standard curve.

The Aflatoxin B<sub>1</sub> Low Matrix Assay is intended for the quantitative detection of aflatoxin B<sub>1</sub> 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 B<sub>1</sub> Low Matrix Microplate (981BAFL01LM): 96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody.
2. Aflatoxin B<sub>1</sub> Standards (983S1BAFL01LM, Black Cap): 6 vials, 1.5 mL/vial of aflatoxin at the following concentrations: 0.0, 0.02, 0.05, 0.1, 0.2, and 0.4 ng/mL in 50% methanol.
3. Aflatoxin B<sub>1</sub>-HRP-Conjugate (984BAFL01LM, Green Cap): 12 mL of aflatoxin B<sub>1</sub> 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. Stop Solution (946P001, 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. Dilution Wells (Red): 96 non-coated wells (12 × 8 well strips) in a microwell holder.

### 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 450 nm
3. Precision pipettes to deliver 100–200 µL volumes
4. Collection Container: Minimum 250 mL capacity
5. Graduated cylinder: 250 mL
6. Hexane, methanol or acetonitrile: 40–200 mL reagent grade per sample
7. Distilled or deionized water
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
12. High-speed, explosion-proof blender (for peanut samples)

### 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 appropriate personal protective equipment (e.g. gloves, 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

Note: For both extraction and assay procedures, bring all reagents to room temperature (19–27 °C) before use.

Do not return unused reagents back into their original bottles.

Before doing the assay, prepare a waste container as a receptacle for the 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<sup>th</sup> 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-dependent)

Note: The sample 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 (95% passes through a 20 mesh screen).
2. Prepare extraction solvent (80% acetonitrile or methanol) 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 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.
6. Dilute an aliquot of the extract 1:10 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 = 1:50

#### *Peanut*

1. Grind a portion of the peanut sample to a paste.
2. Weigh 20 g of the paste into a blender vessel.
3. Add 2.0 g of NaCl.
4. Prepare extraction solvent (80% methanol) by adding 24 mL of distilled or deionized water to 96 mL of methanol for each sample to be tested.
5. Add 120 mL of the 80% methanol extraction solvent to each 20 g paste sample.  
Note: The ratio of sample to extraction solvent is 1:6 (w/v).
6. Add 40 mL of hexane.
7. Blend continuously for 3 minutes with a high-speed, explosion-proof blender (8,000 rpm minimum speed)
8. Filter 10-20 mL of the sample through Whatman #1 filter paper (or equivalent). Collect the filtrate to be tested.
9. Dilute an aliquot of the extract 1:10 with reconstituted PBST wash buffer.
10. The sample is now ready. The standards require no pre-dilution before use.
11. Final dilution for use in calculation = 1:80

*Soy Sauce*

1. 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.
2. Add 20 mL of soy sauce sample to each 100 mL of the 80% acetonitrile extraction solvent.  
Note: The ratio of sample to extraction solvent is 1:5 (v/v).
3. Mix by shaking in a sealed container for a minimum of 5 minutes.
4. Allow the acetonitrile and soy sauce layers to separate. Alternatively, centrifuge a portion of the sample at 3,500 rpm for 5 minutes, to speed the separation. Collect the upper layer, which contains the aflatoxin, to be tested.
5. Dilute an aliquot of the extract 1:10 with reconstituted PBST wash buffer.
6. The sample is now ready. The standards require no pre-dilution before use.
7. Final dilution for use in calculation = 1:50

*Soybean, Chili, Cilantro, and Coriander*

1. Grind a representative sample to the particle size of fine instant coffee (95% 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 100 mL of 80% acetonitrile 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 for a minimum of 5 minutes.
5. Centrifuge the sample at 3,500 rpm for 5 minutes. Alternatively, pass a 5-10 mL portion of the sample through a filter and collect the filtrate to be tested.
6. Dilute an aliquot of the extract 1:10 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 = 1:50

*Corn oil and Peanut oil*

1. Prepare extraction solvent (80% acetonitrile) by adding 40 mL of distilled or deionized water to 160 mL of acetonitrile for each oil sample to be tested.
2. Transfer 200 mL of 80% acetonitrile to a container and add 10 mL of sample.  
Note: The ratio of sample to extraction solvent is 1:20 (v/v).
3. Mix by shaking in a sealed container for a minimum of 30 minutes.

4. Allow the acetonitrile and oil layers to separate. Alternatively, centrifuge a portion of the sample at 3,500 rpm for 5 minutes to speed the separation. Collect the upper layer, which contains the aflatoxin, to be tested.
5. Dilute an aliquot of the extract 1:10 with reconstituted PBST wash buffer.
6. The sample is now ready. The standards require no pre-dilution before use.
7. Final dilution for use in calculation = 1:200

*Safflower oil, Sesame oil, and Vegetable oil*

1. Prepare extraction solvent (80% acetonitrile) by adding 20 mL of distilled or deionized water to 80 mL of acetonitrile for each oil sample to be tested.
2. Transfer 100 mL of 80% acetonitrile to a container and add 10 mL of sample.  
Note: The ratio of sample to extraction solvent is 1:10 (v/v).
3. Mix by shaking in a sealed container for a minimum of 30 minutes.
4. Allow the acetonitrile and oil layer to separate. Alternatively, centrifuge a portion of the sample at 3,500 rpm for 5 minutes to speed the separation. Collect the upper layer, which contains the aflatoxin, to be tested.
5. Dilute an aliquot of the extract 1:10 with reconstituted wash buffer.
6. The sample is now ready. The standards require no pre-dilution before use.
7. Final dilution for use in calculation = 1:100

*Infant and Toddler milk formulas*

1. Prepare extraction solvent (50% methanol) by adding 50 mL of distilled or deionized water to 50 mL of pure methanol.
2. Transfer 100 mL of the 50% methanol to a container and add 20 g of milk formula sample.  
Note: The ratio of sample to extraction solvent is 1:5 (w/v).
3. Mix by shaking in a sealed container for a minimum of 10 minutes.
4. Centrifuge the sample at 3,500 rpm for 5 minutes to pellet the particulate matter.
5. Collect the supernatant, which contains aflatoxin, and proceed to the assay procedures. No further dilution of the sample into wash buffer is necessary.  
Note: Depending on the formulation, some infant formulas will contain a floating fatty layer that must be aspirated. Use the lower plasma layer for the analysis.
6. Final dilution for use in calculation = 1:5

*Rice cereal*

1. Grind a representative rice cereal sample to a fine particle size comparable to powdered sugar. The sample does not need to be passed through a mesh screen.
2. Prepare extraction solvent (50% methanol) by adding 50 mL of distilled or deionized water to 50 mL of pure methanol.
3. Transfer 100 mL of 50% methanol 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 for a minimum of 10 minutes.
5. Centrifuge the sample at 3,500 rpm for 5 minutes to pellet the particulate matter.
6. Collect the supernatant, which contains aflatoxin, and proceed to the assay procedures. No further dilution of the sample into wash buffer is necessary.
7. Final dilution for use in calculation = 1:5

*Animal feed*

1. Grind a representative animal feed sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen).
2. Prepare extraction solvent (80% acetonitrile) by adding 40 mL of distilled or deionized water to 160 mL of acetonitrile for each sample to be tested.
3. Transfer 200 mL of 80% acetonitrile 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 for a minimum of 10 minutes.
5. Centrifuge the sample at 3,500 rpm for 5 minutes to pellet the particulate matter.
6. Collect the supernatant, which contains aflatoxin, for analysis.
7. Dilute an aliquot of the extract 1:10 in reconstituted wash buffer.
8. Final dilution for use in calculation = 1:1000

Assay Procedure

1. Reconstitute the PBST wash buffer to a volume of 1 L with distilled water. The packet contents may be washed out completely with a gentle stream of distilled water. Store the reconstituted PBST 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  $\mu$ L of the Sample Diluent into each mixing well.
4. Using a new pipette tip for each, add 100  $\mu$ L of each Standard and prepared Sample to the appropriate Dilution Well that contains diluent. Mix by priming pipettor at least 3 times.  
Note: Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100  $\mu$ L 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 decant 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 wash buffer.
8. Add 100  $\mu$ L 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  $\mu$ L/well) and place in a separate container. Add 100  $\mu$ L 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  $\mu$ L/well) and place in a separate container. Add 100  $\mu$ L in the same sequence and at the same pace as the Substrate Solution was added.
12. Read and record the optical density (OD) of each microwell with a plate reader, using a 450 nm filter.
13. Setting the zero standard as 100% binding (Bo), calculate the % binding (%B) for each standard and sample as a percentage of the zero binding (%B/Bo).

## Results

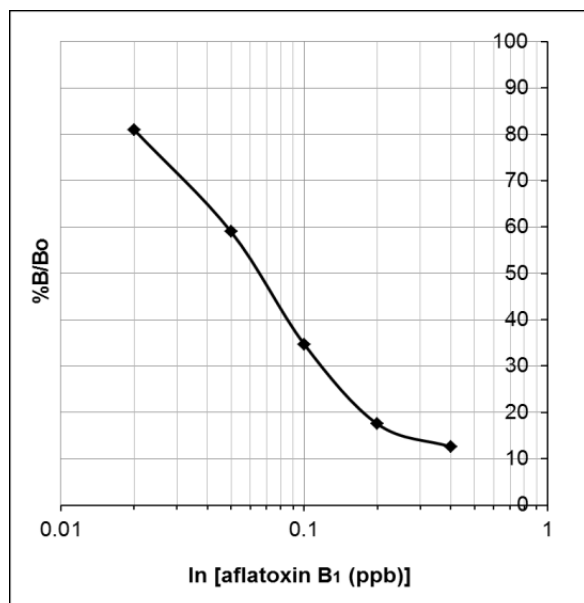
Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/Bo) of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of each standard vial refers to the contents of that vial. However, for any given assay, the sample has been diluted at different ratios depending on the particular protocol, e.g. at a 5:1, 10:1, 20:1, or 100:1 ratio by extraction solvent, as instructed in the Extraction Procedure, and also 10:1 in wash buffer (except no dilution for Infant and Toddler formulas and cereal). Thus the level of aflatoxin shown by the standard must be multiplied by 5, 50, 100, 200, or 1000 in order to indicate the ng per gram (ppb) of the commodity.

The sample dilution results in a standard curve: 0.1–2 ppb, 1–20 ppb, 2–40 ppb, 4–80 ppb, or 20–400 ppb, depending on the dilution factors (see Extraction Procedure section). If a sample contains aflatoxin at greater concentration than the highest standard, it should be diluted appropriately in extraction solvent and retested. The extra dilution step should be factored in when expressing the final result.

### Example of Data

The following standard curve is for demonstration purposes only. Standard curve(s) must be run with each assay.



## Product Profile

% Recovery was determined by spiking various levels (all at parts per billion, ppb) of aflatoxin into the following samples. The extract was prepared either with Acetonitrile or Methanol. Sample % Recovery data are as follows:

### (a) Extraction with 80% Acetonitrile

Sample Type	% Recovery
Soy Sauce (5 ppb)	102
Soybean (5 ppb)	92
Corn oil (20 ppb)	87
Peanut oil (20 ppb)	85
Safflower oil (10 ppb)	93
Sesame oil (10 ppb)	78
Vegetable oil (10 ppb)	93
Grain feed	101
Chili powder (100 ppb)	95.3
Cilantro seed (100 ppb)	95.0
Coriander seed (100 ppb)	101.3

### (b) Extraction with 50% Methanol

Sample Type	% Recovery
Infant milk formula (0.5 ppb)	98
Toddler milk formula (0.5 ppb)	95
Rice cereal	88

Acetonitrile is the preferred extraction solvent. However, methanol may be used if its extraction efficiency is taken into account.

## References

1. Kensler, T.W. *et al.*, Translational strategies for cancer prevention in liver. *Nat. Rev. Cancer*, **3(5)**, 321-329 (2003).
2. Klich, M.A., Environmental and developmental factors influencing by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience*, **48(2)**, 71-80 (2007).
3. 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).

Whatman is a registered trademark of GE Healthcare. TWEEN is a registered trademark of Croda International PLC.

MJM,GA,GCY,PHC,MAM 01/18-2