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Product Information

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

Catalog Number **SE120007**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Aflatoxins are toxic metabolites that different molds like *Aspergillus flavus* and *Aspergillys 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. Aflatoxin B_1 is the most toxic and frequently detected aflatoxin.[1-3]

The Total Aflatoxin ELISA Kit Low Matrix is a solid-phase competitive enzyme immunoassay. An aflatoxin-specific antibody optimized to cross react with all four subtypes of aflatoxin (see cross-reactivity information), 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 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 then 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. 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_{450}). 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.

This Total Aflatoxin Low Matrix ELISA Kit is a competitive enzyme-linked immunoassay intended for the quantitative detection of aflatoxins B_1, B_2, G_1, and G_2 in grains, nuts, cottonseeds, cereals, and other commodities such as silage and most spices, where aflatoxin levels are difficult to measure due to high matrix effects.

Components

1. Aflatoxin Low Matrix Microplate (981AFL01LM): 96 wells (12 × 8 well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody.

2. Aflatoxin Low Matrix Standards (983S1AFL01LM, 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 Low Matrix HRP-Conjugate (984MAFL01LM, 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. 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. Methanol or acetonitrile: 50-200 mL reagent grade per sample
7. Distilled or deionized water (enough for 20-50 mL per sample)
8. Filter Paper: Whatman® #1 or equivalent
9. Filter funnel
10. Absorbent paper towels
11. Centrifuge
12. 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

Note: For both the 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 your 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 1/10th 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: Samples must be collected according to established sampling techniques.

Corn, Wheat, Hay, Snaplage, Paprika, Pistachio, and Peanut

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 or 80% methanol) by adding 20 mL of distilled or deionized water to 80 mL of either acetonitrile or methanol, for each sample to be tested.
3. Transfer 100 mL of 80% acetonitrile or methanol to a container. 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). Collect the filtrate to be tested.
6. Dilute an aliquot of the extract 10-fold with reconstituted wash buffer.
7. The sample is now ready. The standards require no pre-dilution before use.
8. Final dilution for use in calculation = 50-fold

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. Transfer 100 mL of 80% acetonitrile to a container. Add 20 mL of sample.
   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 10-fold 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 = 50-fold

Soy bean, Chili, Cilantro, and Coriander

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 100 mL of 80% acetonitrile to a container. 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 10-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 = 50-fold

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 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 10-fold 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 = 200-fold

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 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 10-fold 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 = 100-fold

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 sample.
   Note: The ratio of sample to extraction solvent is a 1:5 dilution (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 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

Toddler rice cereal

1. Grind a representative 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 a 1:5 dilution (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 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 sample to the particle size of fine instant coffee (50% 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 a 1:100 dilution (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 10-fold in reconstituted PBST wash buffer.
8. Final dilution for use in calculation = 1,000-fold

Assay Procedure

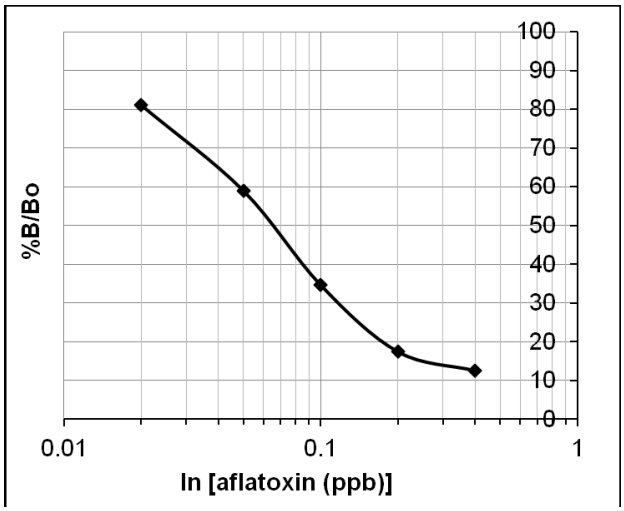
1. Reconstitute the PBS-T packet contents to 1 L volume. The remaining PBS-T powder may be washed out with a gentle stream of distilled water. Refrigerate the reconstituted PBS-T Wash Buffer when not in use.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place 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 mixing well that contains diluent. Mix by priming pipettor at least 3 times.
   Note: The location of each Standard and Sample must be recorded throughout 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 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. Read and record the optical density (OD) of each microwell with a microplate reader using a 450 nm filter..
13. Setting the zero standard as 100% binding (Bo), calculate % 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 with extraction solvent at different ratios depending on the particular protocol, e.g. at a 5:1, 10:1, 20:1, or 100:1 ratio, as instructed in the Extraction Procedure, and also 10-fold with wash buffer (no dilution for baby, toddler formulas and cereal). Thus the level of aflatoxin shown by the standard must be multiplied by 5, 50, 100, 200, or 1,000, 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 Procedures 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 considered when expressing the final result.

Standard Curve Sample Data
The following standard curve, based on data for wheat, is for demonstration only. Standard curve(s) must be run with each assay.



Recovery Sample Data:
Recovery was determined by spiking various levels of aflatoxin into the following samples. Extracts were prepared either with 80% acetonitrile or with 50% methanol as appropriate. % Recoveries are as follows:

1. Samples extracted with 80% acetonitrile (spiked aflatoxin levels given in ppb):

Sample Type	% Recovery
Soy Sauce (5 ppb)	102
Soy bean (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 (100 ppb)	101
Pellet feed (100 ppb)	102
Chili powder (5 ppb)	95.3
Cilantro seed (5 ppb)	95.0
Coriander seed (5 ppb)	101.3

2. Samples extracted with 50% methanol (spiked aflatoxin levels given in ppb):

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

3. Silage samples spiked with 5 ng/g aflatoxin:

   a. Extraction with 80% acetonitrile

Sample Type	% Recovery
Corn	85
Wheat	100
Hay	96
Snaplage	96

   b. Extraction with 80% methanol

Sample Type	% Recovery
Corn	49
Wheat	53
Hay	53
Snaplage	57

4. Other samples spiked with 5 ppb aflatoxin:

   a. Extraction with 80% acetonitrile

Sample Type	% Recovery
Paprika	96
Pistachio	93
Peanut	67

   b. Extraction with 80% methanol

Sample Type	% Recovery
Paprika	67
Pistachio	69
Peanut	58

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

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. Kensler, T.W. et al., Translational strategies for cancer prevention in liver. *Nat. Rev. Cancer*., **3(5)**, 321-329 (2003).
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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).

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