3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Total Aflatoxin ELISA Kit**
**for grains, cereals, nuts, and animal feed**

Catalog Number **SE120006**
Storage Temperature 2–8 °C

# TECHNICAL BULLETIN

## Product Description

The Total Aflatoxin ELISA Kit is a solid-phase direct competitive enzyme immunoassay. An aflatoxin-specific antibody optimized to cross-react with all four subtypes of aflatoxins (see cross-reactivity information) is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated aflatoxin $B_1$ are mixed and added to the antibody-coated microwell. Aflatoxins from the extracted sample and HRP-conjugated aflatoxin $B_1$ compete to bind with the antibody coated to the microwell. Microwell contents are decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added and a blue color develops. The color intensity is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxins in the sample or standard. Therefore, as the concentration of aflatoxins in the sample or standard increases, the intensity of the blue color will decrease.

The Total Aflatoxin Assay 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 including animal feeds.

## Components

1. Aflatoxin Antibody-Coated Microplate (941AFL01M): 96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody.
2. Aflatoxin Standards (943S1AFL01M): 6 vials, 1.5 mL/vial of aflatoxin each at concentrations 0.0, 0.2, 0.5, 1.0, 2.0, and 4.0 ng/mL in 70% methanol.
3. Aflatoxin HRP-Conjugate (944AFL01M; Green Cap): 12 mL of aflatoxin $B_1$ conjugated to peroxidase in buffer with preservative.
4. TMB Substrate (916T001; Blue Cap): 12 mL of stabilized 3,3′,5,5′-tetramethylbenzidine (TMB).
5. Stop Solution (946P001; Red Cap): 12 mL Acidic Solution.
6. Mixing Wells (Green): 96 non-coated wells (12 × 8 well strips) in a microwell holder.
7. Washing Buffer: PBS with 0.05% TWEEN® 20 (PBS-T). Reconstitute to 1 L with distilled water and store refrigerated after reconstitution.

## 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 125 mL capacity
5. Graduated cylinder: 100 mL.
6. Distilled or deionized water
7. Filter Paper: Whatman® #1 or equivalent
8. Filter funnel
9. Absorbent paper towels
10. 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 aflatoxins. Wear appropriate personal protective equipment 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-labeled 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: Bring all reagents to room temperature (19–27 °C) before use.

Extraction
Notes: The sample must be collected according to established sampling techniques.

Samples tested should have a pH of 7.0 ±1.0. Excessive alkaline or acidic conditions may affect the test results.

1. Prepare the Extraction Solution (70% Methanol) by adding 30 mL of distilled or deionized water to 70 mL of methanol (reagent grade) for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
3. Weigh out a 20 g ground portion of the sample and add 100 mL of the Extraction Solvent (70% methanol).
   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. The sample is now ready to be assayed.

Assay
Note: It is recommended that a multichannel pipettor be used to perform the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. The PBS with 0.05% TWEEN® 20 (PBS-T) is reconstituted into a volume of 1 L. The PBS-T packet contents may be washed out with a gentle stream of distilled water.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microplate Wells in another microwell holder.
3. Dispense 200 µL of the HRP-Conjugate into each Dilution Well.
4. Using a new pipette tip for each, add 100 µL of each Standard and Sample to appropriate Dilution Well containing HRP-Conjugate. Mix by priming pipettor at least 3 times.
   Note: Operator must record the location of each Standard and Sample throughout the assay.
5. Using a new pipette tip for each, transfer 100 µL of contents from each Dilution Well to a corresponding Antibody Coated Microplate Well. Incubate at room temperature for 15 minutes.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-T buffer, then decanting the buffer into a discard basin. Repeat wash for a total of 5 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual buffer.
8. Measure the required volume of Substrate Reagent (1 mL/strip or 120 µL/well) and place in a separate container. Add 100 µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
9. 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 was added.
10. Read and record the optical density (OD) of each microwell with a plate reader, using a 450 nm filter.

**Results**
Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0) standard against the aflatoxins content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted at a 5:1 ratio with 70% methanol. Thus the level of aflatoxin shown by the standard must be multiplied by 5 in order to indicate the ng of aflatoxin per gram of commodity (ppb) as follows:

| Standard ng/mL | Commodity (ppb) |
|---|---|
| 0.0 | 0.0 |
| 0.2 | 1.0 |
| 0.5 | 2.5 |
| 1.0 | 5.0 |
| 2.0 | 10.0 |
| 4.0 | 20.0 |

The sample dilution results in a standard curve from 1 ppb to 20 ppb. If a sample contains aflatoxins at greater than the highest standard, it should be diluted appropriately in 70% methanol and retested. The extra dilution step should be factored in when expressing the final result.

Reproducibility:
Intra-Assay: CV <5%
Inter-Assay: CV <8%

Limit of Detection (LOD)
Limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of an aflatoxin-free commodity extract. As different commodities generate somewhat different zeros due to 'matrix inhibition' effects, it follows that the LOD is commodity-specific and should be measured empirically for each different commodity.

Sensitivity
This ELISA kit has been tested at 1 mg/mL (1 million ppb) without evidence of anomalous binding behavior (high-dose hook effect). Therefore, it may be used to assess gross environmental contamination.

Specificity
This assay will cross-react with aflatoxin analogues as follows: $B_1$-100%, $B_2$-77%, $G_1$-64%, $G_2$-25%.

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