



TOTAL AFLATOXIN ASSAY

CAT. NO. 941AFL01M - 96

AFLATOXINS

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four following sub-types of aflatoxin: B₁, B₂, G₁ and G₂. Aflatoxin B₁ is the most toxic and frequently detected form. The other types present a significant danger if the concentration is at a high level. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, decreased milk and egg production. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

INTENDED USE

The HELICA Total Aflatoxin Assay is a competitive enzyme-linked immunoassay intended for the quantitative detection of aflatoxin B₁, B₂, G₁ and G₂ in grains, nuts, cottonseeds, cereals and other commodities including animal feeds.

ASSAY PRINCIPLE

The HELICA Total Aflatoxin Assay is a solid phase direct 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 70% methanol. The extracted sample and HRP-conjugated aflatoxin B₁ are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin B₁ 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 color (blue) develops. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. 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 chromagen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm (OD₄₅₀). The optical densities of the samples are compared to the OD's of the kit standards and an interpretative result is determined.

MATERIALS SUPPLIED

1 pouch: Antibody coated microwells	96 wells (12 eight well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody
1 plate: Dilution wells (green)	96 non-coated wells (12 eight well strips) in a microwell holder
6 vials: Aflatoxin Standards	1.5ml/vial of aflatoxin at the following concentrations: 0.0, 0.2, 0.5, 1.0, 2.0, and 4.0 ng/mL in organic solution
2 bottles: Aflatoxin HRP-conjugate	2 x 12 ml of aflatoxin B ₁ conjugated to peroxidase in buffer with preservative
1 bottle: Substrate Reagent	15ml stabilized tetramethylbenzidine (TMB)
1 bottle: Stop Solution	15ml Acidic Solution

MATERIALS REQUIRED BUT NOT PROVIDED**Extraction Procedure**

Grinder sufficient to render sample to particle size of fine instant coffee

Collection Container: Minimum 125ml capacity

Balance: 20g measuring capability

Graduated cylinder: 100ml

Methanol: 70ml reagent grade per sample

Distilled or deionized water: 30ml per sample

Filter Paper: Whatman #1 or equivalent

Filter Funnel

Assay Procedure

Pipettor with tips: 100µl and 200µl

Timer

Wash bottle

Absorbent paper towels

Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19° - 27°C) before use.
2. Store reagents at 2 to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
4. Adhere to all time and temperature conditions stated in the procedure.
5. Samples tested should have a pH of 7.0 (±1.0). Excessive alkaline or acidic conditions may effect the test results.
6. Never pipette reagents or samples by mouth.
7. Standards are flammable. Caution should be taken in the use and storage of these reagents.
8. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
9. 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.
10. Dispose of all materials, containers and devices in the appropriate receptacle after use.

EXTRACTION PROCEDURE

Note: The sample must be collected according to established sampling techniques

1. Prepare the Extraction Solution (70% Methanol) by adding 30ml of distilled or deionized water to 70ml 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 20g ground portion of the sample and add 100ml 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 - 10ml of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. The sample is now ready for testing.

ASSAY PROCEDURE

Note: It is recommended that a multi-channel pipettor be utilized 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.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter Wells in another microwell holder.
3. Dispense 200 μ l of the Conjugate into each Dilution Well.
4. Using a new pipette tip for each, add 100 μ l of each Standard and Sample to appropriate Dilution Well containing Conjugate. 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 μ l of contents from each Dilution Well to a corresponding Antibody Coated Microtiter 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 distilled or deionized water, then decanting the water 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 water.
8. Measure the required volume of Substrate Reagent (1 ml/strip or 120 μ l/well) and place in a separate container. Add 100 μ l to each microwell. Incubate at room temperature for 5 minutes.
9. Measure the required volume of Stop Solution (1 ml/strip or 120 μ l/well) and place in a separate container. Add 100 μ l in the same sequence and at the same pace as the Substrate was added.
10. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell

INTERPRETATION OF 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 aflatoxin 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, and so 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 1ppb to 20 ppb. If a sample contains aflatoxin at greater than the highest standard, it should be diluted appropriately in 70% methanol and retested. The extra dilution step should be taken into consideration when expressing the final result.

APPENDIX**PERFORMANCE DATA****WITHIN ASSAY VARIATION**

A typical example of the Helica Total Aflatoxin assay run in duplicate yielded the following standard curve and within assay variation.

ppb in sample	Mean OD	CV%
0.0	1.835	1.9
1.0	1.626	1.5
2.5	1.283	1.0
5.0	0.720	2.4
10.0	0.338	1.0
20.0	0.147	1.0

BETWEEN ASSAY VARIATION

Between assay variation is expressed as percentage of Bo for each standard.
n= 6 assays.

ppb in sample	B/Bo%	CV%
1.0	82.6	3.0
2.5	60.6	5.1
5.0	33.6	4.8
10.0	16.6	6.0
20.0	7.7	7.8

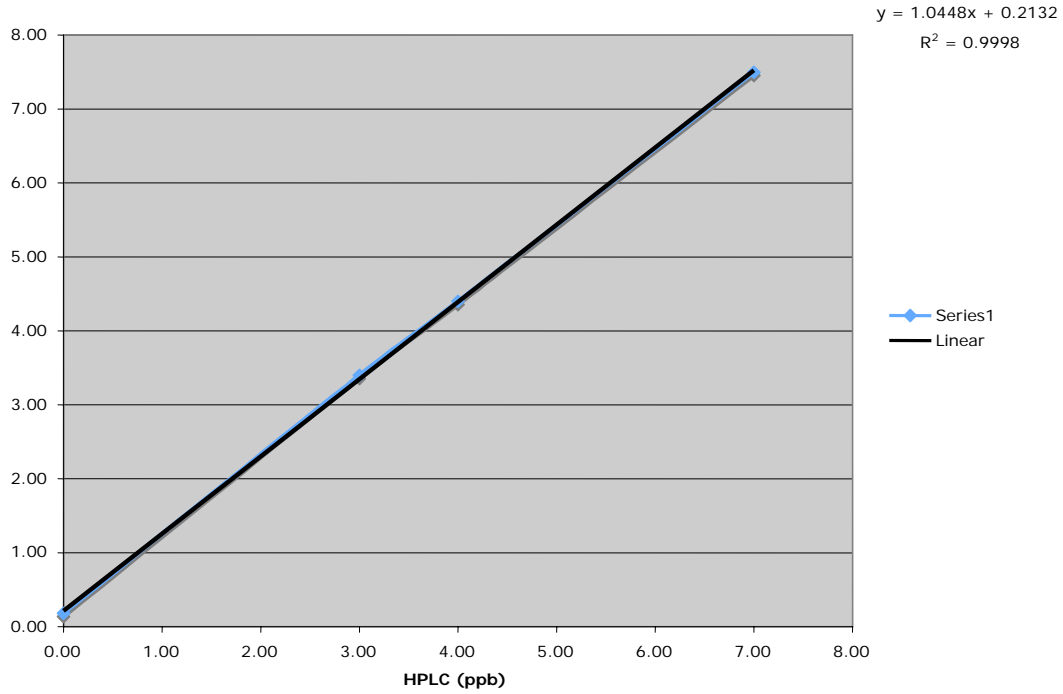
Limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of an Aflatoxin-free commodity extract. As different commodities generate somewhat different zeros due to 'matrix inhibition' effects, it follows that the LOD is commodity specific and should be measured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

Using the Ceres Agrilab Total Aflatoxin assay:

LOD for corn is <1.0 ppb n=10

LOD for peanuts is <1.0 ppb n= 10

Aflatoxin HELICA vs HPLC (maize - corn)



The HELICA Total Aflatoxin ELISA 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.**

CROSS-REACTIONS

The assay will cross-react with aflatoxin analogues as follows:
 B₁-100%, B₂-77%, G₁-64%, G₂-25%

HELICA
 v.02 08-06-05