



## TOTAL AFLATOXIN ASSAY – LOW MATRIX

Cat.No. 981AFL01LM

### **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: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. Aflatoxin B<sub>1</sub> 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 Low Matrix Aflatoxin Assay is a competitive enzyme-linked immunoassay for the quantitative detection of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in grains, nuts, cotton seed, cereals and all commodities which are difficult to measure due to high matrix effect such as silage and most spices.

### **ASSAY PRINCIPLE**

The HELICA Low Matrix 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 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 horse-radish 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, washed and 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. 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 450nm (OD<sub>450</sub>). The optical densities of the samples are compared to the OD's of the kit standards and a result is determined by interpolation from the standard curve.

## Reagents Provided

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: mixing 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, 0.02, 0.05, 0.1, 0.2, and 0.4 ng/mL in aqueous solution
1 bottle: Aflatoxin HRP-conjugate	12 ml of aflatoxin B <sub>1</sub> conjugated to peroxidase in buffer with preservative
2 bottles: Sample Diluent	2 x 12ml propriety sample diluent
1 bottle: Substrate Solution	12ml stabilized tetramethylbenzidine (TMB)
1 bottle: Stop Solution	12ml Acidic Solution
1 pouch: Washing Buffer	PBS with 0.05% Tween20, bring to 1 liter with distilled water and store refrigerated.

## 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 or acetonitrile: 80ml reagent grade per sample.  
Distilled or deionized water: 20ml per sample

Filter Paper: Whatman #1 or equivalent  
Filter Funnel

### Assay Procedure

Pipettor with tips: 100µl and 200µl  
Timer  
Wash bottle  
Dilution tubes  
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. Never pipette reagents or samples by mouth.
6. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
7. 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.
8. 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. 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 20ml of distilled or deionized water to 80% methanol or acetonitrile for each sample to be tested.
3. Weigh out a 20g ground portion of the sample and add 100ml of the Extraction Solvent.  
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.

6. Dilute an aliquot of the extract 1 in 10 with reconstituted wash buffer.
7. The sample is now ready. The standards require no pre-dilution before use.

### **ASSAY PROCEDURE**

1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in use.
2. Place one mixing 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 200µl of the sample diluent into each mixing well.
4. Using a new pipette tip for each, add 100µl of each standard and prepared sample to appropriate mixing well containing 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 100µl of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. 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 PBS-Tween 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 water.
8. Add 100µl of conjugate to each antibody coated well and incubate at ambient temperature for 30 minutes.
9. Repeat steps 6 and 7.
10. Measure the required volume of Substrate Reagent (1 ml/strip or 120 µl/well) and place in a separate container. Add 100µl to each microwell. Incubate at room temperature for 10 minutes.
11. Measure the required volume of Stop Solution (1 ml/strip or 120 µl/well) and place in a separate container. Add 100µl in the same sequence and at the same pace as the Substrate was added.
12. 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.

Note: This assay may be used for detecting aflatoxin in potable water. In this case 100µl of the sample should be used without pre-dilution with wash buffer.

### **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 by extraction solvent and also 10:1 in wash buffer and so the level of aflatoxin shown by the standard must be multiplied by 50 in order to indicate the ng per gram (ppb) of the commodity as follows:

standard ng/mL	commodity (ppb)
0.0	0.0
0.02	1.0
0.05	2.5
0.1	5.0
0.2	10.0
0.4	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 80% extraction solvent and retested. The extra dilution step should be taken into consideration when expressing the final result.

In the case of potable water there is no pre-dilution, so it is measured with a sensitivity equal to the lowest standard which is twenty parts per trillion.

**Assay Characteristics**

Data from ten consecutive standard curves gave the following results:

ppb	B/B <sub>0</sub>	%CV
0	100	-
1	81.0	1.3
2.5	59.0	2.0
5	34.7	5.5
10	17.5	4.9
20	12.6	7.6

As an example of a high matrix effect commodity, thirteen silage samples, 5 corn, 2 wheat, 3 hay and 3 which had measured less than 1 ppb each for B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> by HPLC were extracted with either 80% methanol or 80% acetonitrile.

Following extraction with 80% methanol, 12/13 measured less than 1 ppb, with a single wheat silage sample measuring 1.2ppb. After extraction with 80% acetonitrile, 8/12 measured less than 1 ppb with 5 samples average 1.5 ppb. No sample measured more than 2 ppb.

Recoveries of a 5 ng/gm spike into four of the silage samples were as follows:

	Acetonitrile extract		Methanol extract	
	ppb	% Recovery	ppb	% Recovery
<b>Spike</b>	<b>4.8</b>	<b>100</b>	<b>5.1</b>	<b>100</b>
Corn	4.1	85	2.5	49
Wheat	4.8	100	2.7	53
Hay	4.6	96	2.7	53
Snaplage	4.6	96	2.9	57

In a similar experiment, extraction of paprika, pistachio and peanut by either methanol or acetonitrile was less than 1 ppb and after a 5 ppb spike recoveries were 96%, 93%, and 67% respectively for acetonitrile and 67%, 69% and 58% for methanol.

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

**CROSS-REACTIONS**

The assay will cross-react with aflatoxin analogues as follows:

B<sub>1</sub>-100%, B<sub>2</sub>-77%, G<sub>1</sub>-64%, G<sub>2</sub>-25%

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v.01 07-31-07.