Aflatoxin ELISA Kit

Cat.No: DEIA6846

Lot. No. (See product label)

Pkg#Size

96T

Intended use

The Aflatoxin Plate Kit is a competitive ELISA for the quantitative analysis of aflatoxin in nuts, grain and grain products.

General Description

Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, the most notable ones being Aspergillus flavus and Aspergillus parasiticus. Their name is derived from the early work that discovered Aspergillus Flavustoxins. Aflatoxins are toxic and among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1.

Principle Of The Test

The Aflatoxin kit is a competitive enzyme-labeled immunoassay. Aflatoxin is extracted from a ground sample by blending or shaking with methanol/water. The extract is then diluted with water, filtered and then tested in the immunoassay. Aflatoxin-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Aflatoxin antibody is then pipetted into the test wells to initiate the reaction. During the 10 minute incubation period, aflatoxin from the sample and aflatoxin-HRP enzyme conjugate compete for binding to aflatoxin antibody which, in turn, binds to the test well. Following this 10 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a blue color. Following a 10 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the Aflatoxin concentration of the samples is derived.

Reagents And Materials Provided

1. Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating dessicant.

2 .Chromogen Solution, 1 vail: 12 ml;

3. Standard, 6 vails: 2ml/vail. 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb;

4. HRP-Conjugate,1 vail: 7.0 ml

5. Stop Solution, 1 vail: 10 ml

6. Specimen Diluent(10×),1 vail: 50 ml

7. Wash Buffer(10×), 1vail: 50 ml

8. Instructions

Materials Required But Not Supplied

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1. Laboratory quality distilled or deionized water.

2. Methanol, ACS grade

3. Graduated cylinder, 100 ml or larger.

4. Glassware for sample extraction and extract collection.

5. Filters, Whatman GF/A or equivalent

6. Pipet with disposable tips capable of dispensing 50 μL.

7. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 μL.

8. Paper towels or equivalent absorbent material.

9. Microwell plate or strip reader with 450nm filter.

10. Timer

11. Blender

Storage

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2- 8°C.

Specimen Collection And Handling

1. Grind samples to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.

2. Weigh 50 g ground sample and 5.0 g NaCl and transfer to clean blender jar.

3. Add 100 mL of 80% Methanol/water to the jar.

4. Blend for 1 minute in a high-speed blender.

5. Filter a minimum of 10 mL through a glass fiber filter.

6. Dilute 5 mL of extract with 20 mL of water and mix thoroughly.

7. Filter through a glass fiber filter.

Reagent Preparation

EXTRACTION SOLUTION PREPARATION

1. Carefully measure 20 mL of distilled or deionized water for each 100 mL being prepared and transfer to a clean glass container with tight-fitting lid.

2. Carefully measure 80 mL of Methanol for each 100 mL being prepared and add to the container.

3. Cover and swirl to mix completely. Store tightly sealed to minimize evaporation.

Assay Steps

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1. Allow reagents and sample extracts to reach room temperature prior to running the test.

2. Place the appropriate number of test wells and into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with dessicant.

3. Dispense 50 μL of Enzyme Conjugate into each test well.

4. Using a pipet with disposable tips, add 50 uL of calibrators and samples to the appropriate test wells. Be sure to use a clean pipet tip for each.

5. Dispense 50 μL of Antibody Solution into each test well.

6. Incubate the test wells for 10 minutes.

7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with tap water and dump. Repeat 4X for a total of five washes.

8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.

9. Dispense 100 μL of Substrate into each well.

10. Incubate the wells for 10 minutes.

11. Dispense 100 μL of Stop Solution into each test well.

12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

Typical Standard Curve

The following table is for illustration only. A standard curve must be run with each assay run.

Table 1.

Well content	OD	Mean OD	SD	% RSD	%Bo	Concentration (ppb)
0 ppb	1.773 1.702	1.738	0.050	2.89		
2 ppb	1.320 1.312	1.316	0.006	0.43	75.7	1.9
7.5 ppb	0.825 0.837	0.831	0.008	1.02	47.8	8.2
25.0 ppb	0.464 0.454	0.459	0.007	1.54	26.4	25.4
100.0 ppb	0.187 0.184	0.186	0.002	1.14	10.7	95.9
Sample	0.663 0.706	0.685	0.030	4.44	39.4	12.4

Interpretation of Results

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance’s to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of Aflatoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.

2. Quantitative interpretation requires graphing the absorbances of the calibrators (X axis) versus the log of the calibrator concentration (Y axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the Y axis is the concentration of the sample. Samples with

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absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as 100 ppb, respectively. Alternatively, we can supply a spreadsheet template which can be used for data reduction.

Specificity

The Aflatoxin Plate Kit can’t differentiate between the various Aflatoxins, but detects their presence to differing degrees. The following table shows the relative values for 50% Bo and the (%) cross- reactivity versus Aflatoxin B1. All concentration are in parts per billion (ppb).

Table 2.

Compound	50% Bo (ppb)	Cross-Reactivity (%)
Aflatoxin B1	9.8	100
Aflatoxin B2	39	25
Aflatoxin G1	39.5	25
Aflatoxin G2	221	4

Precautions

1. Each reagent is optimized for use in the Aflatoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Aflatoxin Plate Kits with different Lot numbers.

2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

3. Do not use reagents after expiration date.

4. Reagents should be brought to room temperature, 20-28°C(62-82oF) prior to use. Avoid prolonged (>24 hours) storage at room temperature.

5. Aflatoxin is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All labware should be soaked for at least 1 hour in a 30% solution of household bleach. Avoid contact of skin and mucous membranes with reagents and sample extracts by wearing gloves and protective apparel. If exposure of skin and mucous membranes to liquids should occur, immediately flush with water.

6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes.

Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

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