Beacon Analytical Systems Inc.®

Aflatoxin Plate Kit

Cat. # 20-0017
Product Insert

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081003

PLEASE READ COMPLETELY BEFORE USE

INTENDED USE

The Aflatoxin Plate Kit is a competitive ELISA for the quantitative analysis of aflatoxin in nuts, grain and grain products. The kit has been certified as a Performance Tested Method℠ by the AOAC Research Institute for use in corn and peanuts.

USE PRINCIPLES

The Beacon Aflatoxin Plate Kit is a competitive enzyme-labeled immunoassay. Aflatoxin residues are extracted from a ground sample by shaking or blending with an 80% methanol/water solution. The extract is diluted, filtered and tested in the immunoassay. Aflatoxin-HRP conjugate is pipetted into the test wells followed by sample extract or calibrators. An aflatoxin antibody is added into the test wells to initiate the reaction. During a 10 minute incubation period, aflatoxin from the sample and aflatoxin-HRP conjugate compete for binding to the aflatoxin antibody. Following this incubation, the wells are washed to remove any unbound aflatoxin or aflatoxin-HRP conjugate. After washing, a colorless substrate is added to the wells and any bound aflatoxin-HRP conjugate will convert the substrate to a blue color. Following a 10 minute incubation, the reaction is stopped and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the aflatoxin concentration of the sample is derived. The color intensity is inversely proportional to the amount of aflatoxin present in the sample.

MATERIALS PROVIDED IN THE BEACON AFLATOXIN PLATE KIT

- **Plate** – (1) containing 12 strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.
- **Aflatoxin Calibrators** – (5) vials containing 2 mL each, labeled as 0, 2.0, 7.5, 25 and 100 ppb Aflatoxin. The actual aflatoxin content of each calibrator is 10 fold less than the labeled amount to correspond to the grain sample dilution (10X) employed during the extraction procedure. No further correction is required to obtain the concentration of aflatoxin in the grain sample.
- **Aflatoxin HRP Enzyme Conjugate** – (1) bottle containing 8 mL
- **Aflatoxin Antibody Solution** – (1) bottle containing 8 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL (Caution! Contains 1N HCl. Handle with care.)
- **Product Insert**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE BEACON AFLATOXIN PLATE KIT

Microtiter plate reader or strip reader with 450 nm filter (Stat Fax Model 303 Plus)	Timer
Methanol, ACS grade	Filter paper (coffee filter)
High speed blender	Paper towels or equivalent absorbent material
Pipette with disposable tips capable of dispensing 50 µL	Laboratory quality distilled or deionized water
Multi-channel pipette; 8-channel capable of dispensing 50 and 100 µL	Graduated cylinder, 100 mL or larger
Fisher Scientific G6 or equivalent glass fiber filter	Sodium Chloride (NaCl)

SPECIFICITY: A number of aflatoxin residues can be detected by this assay.
The percent cross reactivity (CR%) of several aflatoxins relative to Aflatoxin B1 is shown in the table below.

Compound	CR%
Aflatoxin B1	**100%**
Aflatoxin B2	**8%**
Aflatoxin G1	**24%**
Aflatoxin G2	**6%**

SENSITIVITY: The limit of detection (LOD) and limit of quantitation (LOQ) for the assay when run with corn and peanuts is shown below.

Matrix	LOD	LOQ
Corn	**0.4 ppb**	**1.2 ppb**
Peanuts	**0.6 ppb**	**1.8 ppb**

KIT HANDLING NOTES and PRECAUTIONS

- Running calibrators and samples in duplicate will improve assay precision and accuracy.
- Reagents should be brought to room temperature, 20 to 28°C (62 to 82°F prior to use). Avoid storing kits for extended periods (>24 hr.) at room temperature.
- The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 to 8°C. The kit expires one year from the date of manufacturing.
- Store all kit components at 2°C to 8°C when not in use. Do not use kit components after the expiration date.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Aflatoxin is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All lab ware should be soaked for at least 1 hour in a 10% 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.
- Do not mix reagents or test strips from kits with different lot numbers or components from any other manufactured kit.
- The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon for technical support if you have any questions about the use of this kit.
- The use of a multichannel pipette to dispense the Enzyme Conjugate, Antibody Solution, Substrate, and Stop Solution is recommended when running 2 strips or more.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- The Stop Solution is 1N hydrochloric acid which is corrosive and an irritant. 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.

EXTRACTION SOLUTION PREPARATION- 80% Methanol/Water

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.

SAMPLE PREPARATION

Corn and other grain(s)

1. Grind samples so that at least 95% of the ground material passes through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not immediately analyzed should be stored refrigerated.
2. Weigh out 50 g of the ground sample and 5.0 g of NaCl and transfer to a 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 through a paper filter (coffee filter is recommended).
6. Dilute 5 mL of the extract with 20 mL of laboratory grade water and mix thoroughly.
7. Filter the diluted extract through a glass fiber filter. The sample is ready for testing in the assay.

Peanut paste

1. Weigh out 50 g of the sample and transfer to a clean blender jar.
2. Add 100 mL of 80% methanol/water to the jar.
3. Blend for 1 minute in a high-speed blender.
4. Filter a minimum of 10 mL through a paper filter (coffee filter is recommended).
5. Dilute 5 mL of the extract with 20 mL of water and mix thoroughly. The sample is ready for testing in the assay.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to room temperature.
2. Remove the required number of test wells from the re-sealable foil bag. Re-seal the remaining strips in the bag containing the desiccant to limit moisture exposure.
3. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
4. Add **50 µL of the Calibrator or Sample Extract** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of the Antibody Solution** into each well. Shake the plate gently to mix contents.
6. Incubate the wells for **10 minutes** at room temperature.
7. After this incubation, decant the contents of the wells into an appropriate waste container. Flood the wells completely with laboratory grade water, then decant. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much of the water wash solution as possible.
8. Add **100 µL of Substrate** to each well. Shake the plate gently to mix contents.
9. Incubate the wells at room temperature for **10 minutes**.
10. Add **100 µL of Stop Solution** to each well. Shake the plate gently to mix contents.
11. Measure and record the absorbance (Optical Density; OD) of each well using a microtiter plate reader at 450nm.

ADDITIONAL DILUTION PROCEDURE

Further dilution is necessary for highly contaminated samples (greater than 100 ppb).

1. Prepare 16% methanol solution by mixing 1 part of the extraction solution (80% methanol/water) with 4 parts of distilled or deionized water. Mix well and store tightly sealed.
2. Dilute the final extract after filtration with this 16% methanol as desired. Run the assay with this diluted extract.
3. Apply the dilution factor to calculate the aflatoxin concentration in the sample.
   If you mixed 1 part of extract with 1 part of 16% methanol, the dilution factor is 2. Multiply the result by 2.
   If you mixed 1 part of extract with 4 parts of 16% methanol, the dilution factor is 5. Multiply the result by 5.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells. Samples with lower absorbance (less color) than a calibrator well, have a concentration of aflatoxin greater than the concentration of the calibrator. Samples with higher absorbance (more color) than a calibrator well, have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbance (OD) from the calibrator wells (Y axis) versus the calibrator concentration (X axis). This can be done using a plate reader with software which uses either a 4-Parameter or Log-Logit curve fit. If your plate reader software does not provide these curve fits, a spreadsheet that will perform the curve fit and sample concentration calculation is available upon request.

SAMPLE CALCULATIONS

Well Contents	Absorbance (OD)	Average OD ± SD*	RSD%	**Bo%	Aflatoxin B1 conc. (ppb)
0 ppb Calibrator	1.773 1.702	1.738 ± 0.050	2.9	100	0
2 ppb Calibrator	1.320 1.312	1.316 ± 0.006	0.4	75.7	1.9
7.5 ppb Calibrator	0.825 0.837	0.831 ± 0.008	1.0	47.8	8.2
25 ppb Calibrator	0.464 0.454	0.459 ± 0.007	1.5	26.4	25.4
100 ppb Calibrator	0.187 0.184	0.186 ± 0.002	1.1	10.7	95.9
Sample	0.663 0.706	0.685 ± 0.030	4.4	39.4	12.4

Actual values may vary; this data is for example purposes only.

* Standard deviation

**Bo% equals the average sample absorbance divided by the average 0 ppb Calibrator absorbance multiplied by 100.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302 or contact us at info@beaconkits.com.

Safety

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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BEACON ANALYTICAL SYSTEMS, INC.®
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302 Fax (207) 602-6502
www.beaconkits.com