Intended Use

The Beacon Aflatoxin Plate Kit is a competitive ELISA for the quantitative analysis of aflatoxin in peanuts and corn. The kit is certified by the Association of Official Analytical Chemists (AOAC) Research Institute under the Performance Tested Methods program (Certificate No. 081003) for the detection of aflatoxin in peanuts and corn.

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 Enzyme Conjugate is pipetted into the Goat α-Rabbit (GAR) coated test wells followed by the Calibrators and the Sample Extract(s). A soluble polyclonal Rabbit α-Aflatoxin antibody solution is then added to the test wells to initiate the reaction. During a 10 minute incubation period, Aflatoxin and Aflatoxin HRP Enzyme Conjugate compete for binding to the Rabbit α-Aflatoxin antibody which is in turn immobilized on the GAR coated test wells. Following the incubation, the wells are washed to remove any unbound Aflatoxin or Aflatoxin HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells, and any bound enzyme conjugate will convert the substrate to a blue color. Following a 10 minute incubation, the reaction is stopped with the addition of Stop Solution 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.

Reagents and Materials Provided

1 Unit	Plate containing 12 strips, each consisting of 8 test wells coated with GAR. The plate is vacuum-sealed in an aluminized pouch with a desiccant.
5 X 2 mL	Vials of Aflatoxin Calibrators (0, 2, 7.5, 25, and 100 ppb). The actual Aflatoxin content of each calibrator is 10 fold less than the labeled amount to correspond to the sample dilution (10X) employed during the extraction procedure. No further correction is required to obtain the concentration of aflatoxin in the sample.
1 X 8 mL	Bottle of Aflatoxin HRP Enzyme Conjugate.
1 X 8 mL	Bottle of Rabbit α-Aflatoxin Antibody.
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for extraction solution preparation.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Enzyme Conjugate, Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Aflatoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Aflatoxin Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Method Performance Claims

Samples used to generate the performance claims were prepared in accordance with the sample preparation protocols described in this brochure.

Matrix	Range (ppb)	LOD (ppb)	LOQ (ppb)	Recovery (%)	RSDr (%)
Peanuts	5 - 48	0.56 - 1.3	1.7 - 3.9	73.0 - 122	4.47 - 21.4
Corn	5 - 112	0.39	1.2	71.9 - 101*	6.15 - 15.4*

* Data reflect intermediate precision and recovery.

LOD = Limit of Detection
LOQ = Limit of Quantitation
RSDr = Repeatability Relative Standard Deviation
1 ppb = 1 µg/Kg

Method Selectivity

Compound	Concentration (µg/Kg)	% Cross-Reactivity
Aflatoxin B1	5	100
Aflatoxin B2	5	8
Aflatoxin G1	5	24
Aflatoxin G2	5	6

Extraction Solution Preparation (80% Methanol)

1. Measure 20 mL of distilled or deionized water for each 100 mL being prepared and transfer to a clean glass container with a tight-fitting lid.
2. Measure 80 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

Sample Preparation

Peanut paste:

1. Weigh 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.

Corn:

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 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.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50 µL of Enzyme Conjugate** into each well.
4. Dispense **50 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of Antibody** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **10 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **10 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used wells in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Aflatoxin in a sample is determined by comparing the average sample absorbance to the standard curve.
- In the event that the average absorbance of the sample is lower than the highest calibrator, follow the additional dilution procedure.

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 laboratory quality 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.

Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

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

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