CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance's to the absorbance of the calibrator tubes: 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 (Y axis) versus the log of the calibrator concentration (X 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 absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <2 ppb or >100 ppb, respectively. A spreadsheet that will perform the curve fit and sample calculations is available on our website, www.beaconkits.com or can be provided upon request.

SAMPLE CALCULATIONS

Well Contents	OD		Mean OD	SD*	%RSD	%Bo**	ppb Afb1
0 ppb	1.773	1.702	**1.738**	0.050	2.89		
2 ppb	1.32	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 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**

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

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material 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.

Aflatoxin Tube Kit

Cat.# 20-0099

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

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

USE PRINCIPLES

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

MATERIALS PROVIDED IN THE BEACON AFLATOXIN TUBE KIT

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

- 40 (100) Anti–Rabbit IgG coated test tubes vacuum-packed in aluminized pouch with indicating desiccant.
- 5 vials, each containing 5 (10) mL of Aflatoxin calibrators corresponding to 0, 2.0, 7.5, 25 and 100 µg/L (ppb) of Aflatoxin. (Note: Because of the 1:10 dilution of the grain sample in the extraction step, the calibrators actually contain 1/10th of the stated value. No further correction back to the concentration in the original grain sample is required.)
- 1 vial containing 24 (60) mL of Aflatoxin-HRP Enzyme Conjugate.
- 1 vial containing 24 (60) mL of anti-Aflatoxin antibody.
- 1 vial containing 24 (60) mL of Substrate.
- 1 vial containing 24 (60) mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Aflatoxin Tube Kit cannot differentiate between the various Aflatoxins, but detects their presence to differing degrees. The following table shows the relative values for 50% B_o and the % cross reactivity versus Aflatoxin b1. All concentrations are in parts per billion (ppb).

COMPOUND	50% BO	% CR
AFLATOXIN B2	39	25%
AFLATOXIN G1	39.5	25%
AFLATOXIN G2	221	4%

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Methanol, ACS grade
- Sodium chloride, Reagent grade.
- Graduated cylinder, 100 ml or larger.
- High speed blender.
- Glassware for sample extraction and extract collection.
- Filter paper(coffee filter).
- Whatman GF/A or equivalent glass fiber filter.
- Pipette with disposable tips capable of dispensing 500 µL.
- Paper towels or equivalent absorbent material.
- Photometer capable of reading 12mm tubes at 450nm.
- Timer.
- Balance.

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Aflatoxin Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Aflatoxin Tube 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 – 28ºC (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 lab ware 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.

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.

SAMPLE PREPARATION

Corn and other grains

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 paper filter (paper coffee filter is recommended).
6. Dilute 5 mL of extract with 20 mL of water and mix thoroughly.
7. Filter through a glass fiber filter.

Peanut paste

1. Weigh 50 g sample and transfer to a blender jar or other appropriately sized container with tight fitting lid (250 mL).
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 (paper coffee filter is recommended).
5. Dilute 5 mL of extract with 20 mL of water and mix thoroughly. This solution can be assayed without filtering.

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test tubes into a tube holder. Be sure to re-seal unused tubes in the zip-lock bag with desiccant.
3. Dispense **500 µL of Enzyme Conjugate** into each test tube.
4. Using a pipette with disposable tips, add **500 µL of calibrators and samples** to the appropriate test tubes. Be sure to use a clean pipet tip for each.
5. Dispense **500 µL of Antibody Solution** into each test well.
6. Shake the tube rack gently to mix contents, incubate the test tubes for **10 minutes**.
7. Dump the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with Laboratory quality distilled or deionized water and dump. Repeat 4X for a total of five washes.
8. Following the last wash tap the inverted tubes onto absorbent paper to remove the last of the wash solution.
9. Dispense **500 µL of Substrate** into each well.
10. Shake the tube rack gently. Incubate the tubes for **10 minutes**.
11. Dispense **500 µL of Stop Solution** into each test well. Shake the tube rack gently to mix.
12. Read and record the absorbance of the tubes at 450nm using a strip or Tube reader.