PRECAUTIONS

1. Each reagent is optimized for use in the Quan-Tox Aflatoxin Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Quan-Tox Aflatoxin Tube Kits with different lot numbers.
2. A 50 g subsample is required for proper extract preparation.
3. Follow prescribed pipetting and shaking methods.
4. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
5. Do not use reagents after expiration date.
6. Kits should be stored refrigerated at 4-8 ºC. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (>24 hours) storage at room temperature. High room temperature storage (> 28ºC) may cause inaccurate results.
7. Aflatoxin is a toxic substance. Dispose of all liquids in a plastic container containing 30% household bleach. All lab ware should be soaked for at least 1 hour in bleach solution. 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.
8. 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.

Notes:

1. Samples with concentrations less than the lowest calibrator or greater than the highest calibrator must be reported as < 2 ppb or >100 ppb, respectively.
2. Microsoft Excel software is recommended to run the spreadsheet. The following are alternatives to Microsoft Excel:

- OpenOffice: Free software available at www.openoffice.org
- Google Docs: Free web-based software available after open a Google account (email account).

Example of Typical Calibrator B/Bo% Values[1]

Calibrator concentration	B/Bo % Range
0 ppb	**100**
2 ppb	**73.1 - 77.3**
7.5 ppb	**41.2 – 46.3**
25 ppb	**18.9 – 21.3**
100 ppb	**7.3 – 9.81**

[1]These ranges are examples only.

For questions regarding this kit or for additional information about Beacon products, contact your local distributor.

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.

Quan-Tox Aflatoxin Tube Kit

Cat. # 20-0230

Lot # 000000

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

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

USE PRINCIPLES

The Quan-Tox Aflatoxin Tube Kit is a competitive enzyme-labeled immunoassay used for quantitation of aflatoxin from grain and grain products. Aflatoxin residues are extracted from a ground samples by blending with a methanol/water mixture. Sample extract is diluted with water, filtered, and then tested in the immunoassay. Aflatoxin-HRP enzyme conjugate is pipetted into the test tubes followed by calibrators, controls 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 aflatoxin antibody which, in turn, binds to the test tube. Following the incubation, the contents of the tube are washed to remove any unbound materials. A clear substrate is then added to the tube(s) and any bound aflatoxin-HRP conjugate will react by converting to a blue color. Following a 10 minute incubation, the reaction is stopped and amount of color in each tube is measured. The color intensities of unknown sample and calibrators are measured at 450 nm. The ratio of sample to zero calibrator is calculated (%B/Bo), and the aflatoxin concentration of the sample is derived from the calibration curve.

MATERIALS PROVIDED IN THE 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 coated test tubes vacuum-packed in aluminized pouch with indicating desiccant.
- 1 vial containing 8 mL Negative Control.
- 1 vial containing 8 mL Positive Control (10 ppb).
- 5 vials containing Calibrators corresponding to 0, 2, 7.5, 25 and 100 ppb Aflatoxin*.
- 1 vial containing 24 mL of Aflatoxin-HRP Enzyme Conjugate.
- 1 vial containing 24 mL of anti-Aflatoxin antibody.
- 1 vial containing 24 mL of Substrate.
- 1 vial containing 24 mL of Stop Solution.
- Instructional booklet and Data Reduction Spreadsheet.

*Actual aflatoxin concentration in Calibrators is 10% of the stated value to compensate for the sample extraction dilution factor of 1:10.

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Quan-Tox Aflatoxin Tube Kit measures the total amount of aflatoxin residues present in the sample extract. The specificity to other aflatoxins is demonstrated by measuring the cross reactivity versus Aflatoxin B1 as shown below.

Compound	% Cross Reactivity
Aflatoxin B2	28
Aflatoxin G1	20
Aflatoxin G2	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.
- Filter paper (coffee filter).
- Glass fiber filter (Fisher Scientific G6 or equivalent).
- Pipette with disposable tips to dispense 500 µL (0.5 mL).
- Photometer capable of reading 12mm tubes at 450nm (Pocket Colorimeter II).
- Timer, blender (Oster), blender jars (8 oz. jar), and balance.

CALCULATION OF RESULTS

SECTION 1

When running a test kit for the first time, the complete calibration curve must be generated along with the test sample(s) and Positive Control. Select 6 tubes, one for each Calibrator (0, 2, 7.5, 25, 100 ppb), Positive Control (10 ppb) and a tube for each sample extract to be tested (Due to the time intervals between tubes, use up to 8 tubes per assay). Insert absorbance values into **Section 1** of the spreadsheet to calculate the results.

SECTION 2**

When running additional samples with the test kit after you have generated the previous kit calibration curve, you only need to run a Negative and Positive Control alongside your sample extracts. For example – to test one sample you will need 3 tubes Negative Control, Positive Control and sample.

1. Follow **ASSAY PROCEDURE**.
2. Insert absorbance values of Negative Control, Positive Control and sample in **Section 2** of the spreadsheet to calculate the results.
3. Verify Positive Control is within a valid error range of ±30% (7 – 13 ppb). If the Positive Control falls within the range, the sample result is valid. If not, re-run the **calibration curve only** and insert the absorbance values into **Section 1** of the spreadsheet to generate an updated standard curve.
4. Sample result(s) in **Section 2** will automatically update to give the resulting aflatoxin levels.

**It is not necessary to rerun the complete calibration curve if the positive control values fall within the expected range. It is recommended to run a new calibration curve after long term storage (>1 mo.) or if kit has been stored above the recommended refrigerated storage temperature.

EXTRACTION SOLUTION PREPARATION

1. To prepare 80% methanol, mix 8 parts ACS grade methanol with 2 parts distilled or deionized water.
2. Store tightly sealed to minimize evaporation.

SAMPLE PREPARATION (For nuts, grains and grain products)

1. Grind samples to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not immediately analyzed should be stored refrigerated.
2. Weigh **50 g** ground sample and 5.0 g 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** a minimum of 10 mL through a paper filter (paper coffee filter is recommended).
6. **Dilute extract 1:5** with DI water (5 mL + 20 mL water). Mix for 30 seconds.
7. **Filter** through a glass fiber filter.
8. Use filtrate for the assay.

ASSAY PROCEDURE

(When adding reagents or samples to the tubes, hold the pipette vertically and dispense directly above the solution.)

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 aluminized pouch bag with desiccant.
3. **Add 500 µL of Enzyme Conjugate** into each test tube.
4. **Add 500 µL Calibrators, Controls or samples** into each tube using a clean tip for each addition.
5. **Add 500 µL of Antibody Solution** into each test tube.
6. Following prescribed shaking method, shake the tube rack for 20 seconds to mix contents, **incubate 10 minutes**. Shaking Method – Tube rack should remain on table and move right to left to mix contents gently. Do not lift rack and shake.
7. **Wash 5 times** by dumping all of the tube contents into an appropriate waste container and filling tubes to overflowing 5 times with laboratory quality distilled or deionized water.
8. Following the last wash, forcefully flick the inverted tubes several times to remove the last of the wash solution.
9. **Add 500 µL of Substrate** into each tube.
10. Shake the tube rack for 20 seconds and **incubate 10 minutes**.
11. **Add 500 µL of Stop Solution** into each tube. Shake the tube rack gently to mix.
12. Read and record the absorbance of the tubes at 450nm using a Pocket Colorimeter II.
13. Insert absorbance values of Calibrators, Controls or samples to spreadsheet. Refer to **CALCULATION OF RESULTS**