

Aflatoxin Tube Kit – PN 53011B

Intended Use

The Aflatoxin Tube Kit is a competitive ELISA for the quantitative analysis of aflatoxin in corn and peanuts.

Assay Principles

The Aflatoxin Tube Kit is a competitive enzyme-labeled immunoassay. Aflatoxin is extracted from a ground sample by shaking with a methanol/water mixture. The extract is diluted with water and filtered and then is tested in the immunoassay. The sample extract is mixed with the enzyme-labeled aflatoxin and specific aflatoxin antibodies in a test tube. The toxin from the extract and the enzyme-labeled toxin compete for a limited number of antibody binding sites. Following this 10 minute incubation, the contents of the tubes 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.

REAGENTS AND MATERIALS PROVIDED

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.

1. 40 Goat anti-Rabbit IgG coated test tubes in a sealed foil pouch with indicating desiccant.
2. 5 vials each containing 7 mL of aflatoxin calibrators corresponding to 0, 2.0, 7.5, 25 and 100 µg/kg (ppb) of aflatoxin in a 16% methanol solution. (Note: Because of the 1:10 dilution of the 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 sample is required.)
3. 1 vial containing 24 mL of aflatoxin-HRP Enzyme Conjugate.
4. 1 vial containing 24 mL of anti-Aflatoxin antibodies.
5. 1 amber vial containing 24 mL of Substrate.
6. 1 vial containing 24 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
7. Instructions

PRECAUTIONS

1. Each reagent is optimized for use in the Aflatoxin Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other 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 (62-82°F) 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory quality distilled or deionized water.
2. 80% Methanol Extraction Solvent. (Mix 8 parts reagent grade methanol with 2 parts lab quality water.)
3. Sodium Chloride, Reagent grade.
4. Graduated cylinder.
5. High-speed blender.
6. Glass fiber filters, Whatman GF/A or equivalent
7. Pipet with disposable tips capable of dispensing 500 µL.
8. Paper towels or equivalent absorbent material.
9. Photometer capable of reading 12mm tubes at 450nm.
10. Timer
11. Balance

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

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. Prepare extraction solvent by mixing 80 mL Methanol with 20 mL water.
3. Weigh 50 g ground sample and 5.0 g NaCl and transfer to clean blender jar.
4. 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 glass fiber filter.
5. Dilute 5 mL of extract with 20 mL of water and mix thoroughly.
6. Filter through a glass fiber filter.

TEST PROCEDURE

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of antibody-coated tubes into the tube holder. Be sure to re-seal unused tubes in the zip-lock pouch with dessicant.
3. Dispense **500 μ L of Enzyme Conjugate** into each tube.
4. Dispense **500 μ L of each Calibrator and Sample Extract** into the appropriate tubes. Use a clean pipet tip for each.
5. Dispense **500 μ L of Antibody** into each tube.
5. Shake the rack vigorously to mix the contents of the tubes.
7. Incubate the tubes for **10 minutes**.
8. Dump the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with laboratory grade water and dump wash. Repeat 4X for a total of five washes.
9. Following the last wash tap the inverted tubes onto absorbent paper to remove the last of the wash.
10. Dispense **500 μ L of Substrate** into each tube.
11. Incubate the tubes for **10 minutes**.
12. Dispense **500 μ L of Stop Solution** into each test tube.
13. Read and record the absorbance of the tubes at 450nm.

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator tubes: Samples containing less color than a calibrator tube have a concentration of aflatoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator tube 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 absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 1 ppb or >25 ppb, respectively.

Alternatively, Abraxis can supply a spreadsheet template which can be used for data reduction. Please contact Abraxis for further details