Importance of Aflatoxin Determination

Aflatoxins are highly toxic mycotoxins produced by a variety of molds such as *Aspergillus flavus, A. parasiticus* and *A. nomius.* The toxins most frequently detected are Aflatoxin B_1, B_2, G_1, and G_2. Crops may be contaminated during growth, harvest or storage. These toxins are known carcinogens and can be present in grains, nuts, cottonseed and other foods consumed by humans or in animal feed. When animals are fed contaminated feed, Aflatoxin B_1 is converted to M_1 by hydroxylation and is subsequently secreted into the milk of lactating animals. Aflatoxin M_1 is very stable throughout milk processing methods such as pasteurazation. Human breast milk can also contain Aflatoxin M_1 if a lactating woman has consumed food contaminated with Aflatoxin B_1.

To protect humans, regulatory agencies around the world have imposed regulatory limits regarding the amount of Aflatoxins that are allowable in human and animal foods. The maximum Aflatoxin levels as follows:

Grains: European Union (EU) - B_1 = 2-4 ppb; USA – total aflatoxins = 20 ppb.
Corn: EU - B_1 = 5 ppb, total aflatoxins = 10 ppb; USA – total aflatoxins = 20 ppb.

Performance Data

Sensitivity:

Sample	Cut-off
Wheat	5 ppb
Corn	5 ppb

Specificity: The Abraxis Aflatoxin Strip Test was evaluated to detect various Aflatoxins (B_1, B_2, G_1, and G_2).

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

Additional Ordering Information:

Aflatoxin ELISA Microtiter Plate (96T) PN 53012B
Aflatoxin M1 ELISA Microtiter Plate (96T) PN 53012M
Aflatoxin M1 Strip Test in Milk and Infant Formula Samples PN 53012A

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Aflatoxin Strip Test

A Screening Test for Rapid Detection of Aflatoxin in Wheat and Corn Samples

Product No. 53212AB

1. General Description

The Abraxis Aflatoxin Strip Test is designed solely for the preliminary screening of contaminant samples (please contact Abraxis for food matrix of interest). This test is suitable for the qualitative screening of Aflatoxin in wheat and corn at 5 ppb (see Sample Preparation, Section D). The Aflatoxin Strip Test provides preliminary qualitative test results only. Other conventional methods, such as ELISA, HPLC, or GC/MS, should be used to obtain quantitative results or to confirm positive samples.

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Aflatoxin Strip Test components and reagents should be stored at room temperature (20-30°C). Protect from light and moisture. Reagents may be used until the expiration date on the box.

4. Test Principle

The Aflatoxin Test Strip consists of a membrane strip containing an Aflatoxin conjugate. A Control Line, produced by a different antibody-antigen reaction, is also present on the membrane strip. Aflatoxin, if present in a sample solution, will bind with gold-labeled antibodies in the sample pad, forming an antibody-antigen complex. The solution wicks up causing the fluid to pass over an area containing the immoblized aflatoxin conjugate specific to the gold-labeled antibodies on the nitrocellulose membrane (test line). The excess unbound gold-labeled antibodies will continue to migrate up the strip and bind to different immobilized antibodies specific to the gold-labeled antibody, producing a second visible line (control line). In the absence of Aflatoxin in the sample or extract, the colloidal gold labeled antibody contacts the immobilized Aflatoxin conjugate on the strip. An antibody-antigen reaction occurs forming a visible line. The Control Line is not influenced by the presence or absence of Aflatoxin sample or extract, and therefore, should be present in all reactions. The formation of two visible lines indicates a negative result (below the detection limit or cut-off). If Aflatoxin is present in the sample or extract, it competes with the immobilized Aflatoxin conjugate in the test area for binding sites on the colloidal gold labeled antibody. If a sufficient amount of Aflatoxin is present in the sample or extract, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the immobilized Aflatoxin conjugate and, therefore no line will develop. If a colored line is not visible in the Test Line region, or if the Test Line is significantly lighter than the Control Line, the sample or extract is positive (above the detection limit or cut-off).

5. Limitations of the Aflatoxin Strip Test

The Aflatoxin Strip Test is designed for use with contaminant samples. Samples must be prepared as instructed in the Sample Preparation Section (Section D).

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test strip or reagents, inaccurate volumes of sample, extract or reagents, too long or too short incubation times, and extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis Aflatoxin Strip Test provides preliminary qualitative screening results. Reasonable judgment should be applied to any test results, particularly when preliminary positive results are obtained. Other conventional methods, such as ELISA, HPLC, or GC/MS should be used to obtain quantitative results or to confirm positive samples.

A. Warnings and Precautions
1. Prior to use ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
2. Use reasonable judgment when interpreting results.
3. As the sample or extract runs through the test strip, the membrane may become tinted pink. This does not invalidate the test or cause inaccurate results.

B. Reagents and Materials Provided
1. Two (2) canisters containing 25 strips (50 strips total).

C. Additional Materials (not provided)
1. Timer
2. Vortex mixer

D. Sample Preparation
1. *Wheat and Corn* – 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 1 g ground sample and 0.2 g Sodium Chloride in 15 mL conical vial.
3. Add 5 mL of 60% methanol/water to samples.
4. Vortex or shake for 1 minute. Let sediments settle for at least 2 minutes.
5. *Dilute extract 4-fold by removing 60 µL extract to 180 µL PBS in 1.5 mL or 2.0 mL microcentrifuge vial.
6. Proceed to Strip Test Procedure, Section G.

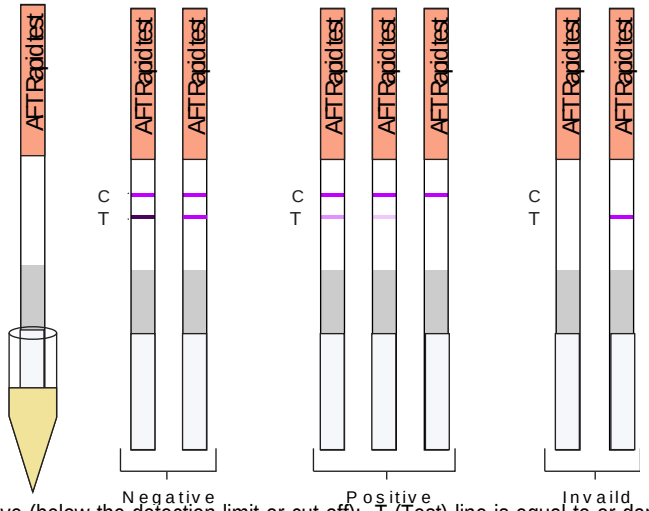
*Note: Diluted extract must contain 20% or less methanol concentration in sample.

E. Additional Reagents and Materials (for Sample Extraction, not provided)
1. Deionized or distilled water
2. Sodium Chloride
3. Methanol
4. Phosphate Buffered Saline (PBS)
5. 15 mL or 50 mL conical vials
6. 1.5 mL or 2.0 mL microcentrifuge vials
7. Serological pipettes, 5 mL or 10 mL
8. Balance with 0.01 g accuracy
9. Centrifuge capable of 3000 rpm (1700 x g) (optional)
10. Micropipette (200 µL,1000 µL) with disposable tips

F. Prepare Methanol Extraction Solution
1. Prepare 1000 mL of 60% methanol by adding 600 mL methanol to 400 mL deionized or distilled water.

G. Strip Test Procedure
1. Prepare sample according to Sample Preparation instructions (Section D).
2. Open the canister containing the strips. Remove the required number of strips.
3. Holding the strip vertically, immerse the end of the strip into the sample liquid.
4. Take the strip out when the sample has completely migrated to the test window (about 10 seconds). Lay the strip facing up flat on a clean, dry, non-absorbent surface.
5. Interpret the results ***within 10 minutes*** according to the Interpretation of Results criteria (Section H).

H. Interpretation of Results



1. Negative (below the detection limit or cut-off): T (Test) line is equal to or darker than C (Control) line.
2. Positive (above the detection limit or cut-off): T line is not present or significantly lighter than C line.
3. Invalid Test: C line is not present. Retest using another aliquot of sample or extract with new microtiter well and test strip.

Note: Illustration is for demonstration of test line intensity range only, since overall intensity may vary slightly with different lots of reagents, samples, extracts, etc. Results should be determined ***within 10 minutes*** after completion of the strip test procedure. Interpretation of the results beyond the 10 minutes may produce inaccurate results.