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Product Manual

AgraStrip® Total Aflatoxin Test (10 pb Cutoff)
Part #10002131

Intended use
The AgraStrip® Aflatoxin Test is a one-step lateral flow immunochromatographic assay that determines a qualitative level for the presence of total aflatoxin (B1, B2, G1 and G2) and is intended for use in grains, cereals and other commodities.

The AgraStrip® Aflatoxin Test has been validated for a variety of food products, such as corn, peanuts and almond, among others.

Aflatoxins
Aflatoxins are highly toxic and carcinogenic compounds produced as metabolites by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Four principal types of aflatoxins are B1, B2, G1, and G2, each named for their distinct fluorescent properties. Aflatoxin B1 is not only the most common but also the most toxic of these compounds. These toxins are predominantly found in commodities such as cereals, corn, peanuts, cottonseed, and nuts. In animals, aflatoxins can cause liver disease and lead to decreased productivity, affecting milk production, egg production, and weight gain. Aflatoxin B1 is classified as a potent human carcinogen and is associated with an increased risk of liver cancer.

Assay Principles
The AgraStrip® Aflatoxin Test Method is a one-step lateral flow immunochromatographic assay with a cutoff level of 10 ppb aflatoxin based on an inhibition immunoassay format. Antibody-particle complex is dissolved in assay diluent and mixed with sample extract. The mixed content is then wicked onto a membrane, which contains a test zone and a control zone. The test zone captures free antibody-particle complex, allowing color particles to concentrate and form a visible line. A positive sample with aflatoxin above the cutoff level will result in no visual line in the test zone. Alternatively, a negative sample with aflatoxin below the cutoff level will form a visible line in the test zone. The line will always be visible in the control zone regardless of the presence of aflatoxin.

Precautions

1. Store test kits at 15-25°C (59-77°F) when not in use, and do not use beyond the expiration date.
2. Test strips and microwells must be kept inside their original tubes.
3. All reagents must be at room temperature before assay is running.
4. Adhere to the instructions of test procedures.
5. Do not re-use test strips.
6. Methanol is flammable. Caution must be taken in its use and storage.
7. Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
8. The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

Procedure

Sample Preparation / Extraction

1. Obtain a representative sample and grind it using a mill so that 95% will pass through a 20-mesh sieve (sieve opening 0.84mm), then thoroughly mix the subsample portion.
2. Weigh out 10 g of ground sample into a clean jar that can be tightly sealed.
3. Add 20 mL of 70% methanol extraction solution (i.e. 70/30 (v/v) methanol/water) or 20 mL of 50% ethanol extraction solution (i.e. 50/50 (v/v) ethanol/water) and seal jar.

Note: Samples should be extracted in a ratio of 1:2 (w:v) of sample to extraction solution respectively.

4. Vigorously shake, blend or vortex for 1 minute.
5. Allow sample to settle or filter the top layer of extract through a Whatman #1 filter and collect the filtrate. Proceed to the test procedure.

Note: Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

Test Procedure

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use.

1. Remove microwell sealer and place the appropriate number of microwells in a microwell holder. Re-seal those un-used microwells.
2. Using a single channel pipette, add 50 µL of assay diluent to each microwell. Dissolve the coating conjugate in the microwell by pipetting the content up and down 5 times.
3. Add 50 µL of sample extracts to each microwell, mixing the content in each well by pipetting it up and down 3 times.
4. Put one test strip in one well.
5. Allow the test strip to develop color for 5 minutes.
6. Interpret test results immediately.

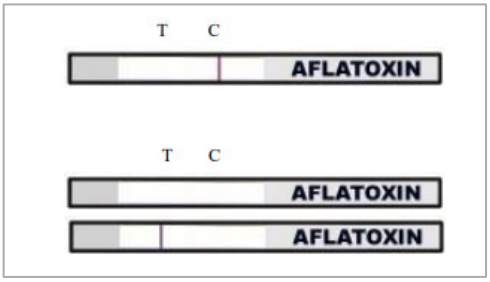
Interpretation of the Results

A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test results. This line is the Test Line (T).

Results less than 10 ppb: 2 lines are visible. This indicates that the sample contains total aflatoxin less than 10 ppb (negative sample). A negative sample may be determined as soon as 2 lines are visible on the strip. (Please see right-hand side image)

Results greater than 10 ppb or equal to 10 ppb: 1 line is visible. This indicates that the sample contains total aflatoxin greater than or equal to 10 ppb (positive sample). (Please see right-hand side image).

Invalid results: If there is no line in control zone, the test is invalid, and the sample should be re-tested by using a valid test strip. (Please see right-hand side image)



NOTE: For some samples, a third line may appear on the strip at a position of approximately 0.4 cm from the bottom portion of the AgraStrip®. Please note that if the line at the bottom of the strip appears, it is not a valid line and can be ignored. Only the Test line and the Control line, which correspond to the letters "T" and "C" on the back of strip respectively, are used in interpreting results. The Control line must always be present for the test to be valid.

Performance Characteristics

AgraStrip® Total Aflatoxin Test has been validated to screen for total aflatoxin in corn, peanuts and almond at the 10 ppb cutoff level

Materials Supplied with Kit

- 1 tube containing 24 aflatoxin test strips
- 1 tube containing 24 microtiter wells coated with antibody-particle complex
- 1 bottle of 1.7 mL assay diluents
- 1 piece of microwell holder
- 1 bag of 48 pieces of pipette tips

Materials Required but not Provided with Kit

Extraction Procedure

- Romer Series II® Mill or equivalent
- Blender or a tightly sealing jar with lid
- Analytical balance with a weighing capacity up to 400 g
- Graduated cylinder (10002612) with a minimum capacity of 100 mL
- 70% methanol or
  - ACS grade methanol for making 70% methanol
  - Distilled or de-ionized water for making 70% methanol

- 50% ethanol or
  - Formula 3A ethanol for making 50% ethanol
  - Distilled or de-ionized water for making 50% ethanol
- Container with a minimum 50 mL capacity
- Whatman#1 filter paper, or equivalent (optional)
- Filter funnel (optional)

Assay Procedure

- Single channel pipettor capable of pipetting 50 µL with tips
- Timer

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