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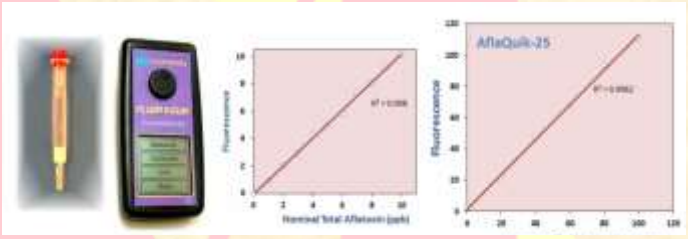
Aflatoxin Rapid Detection System Using Handheld Fluorometer

Description:

Aflatoxins are naturally occurring fungal toxins that can be present in many different types of food. Contamination of food with aflatoxin can cause a variety of illnesses including liver cancer. The Total Aflatoxin Assay Kit contains immunoaffinity columns and reagents for the convenient purification, enrichment, and quantitation of total aflatoxin (B1, B2, G1, and G2) from a variety of food and feed samples. The columns contain immobilized antibodies which bind all aflatoxins. When food sample extracts are passed through the columns, aflatoxins will specifically bind to the column while the other substances pass through. The Derivitizing Reagent and Total Aflatoxin Standard allow this purified sample to be directly measured using fluorometer operating at 360ex/460em.

Assay Performance:

- Rapid (25min), convenient, sensitive, and accurate compared to most methods based on ELISA.
- High portability using handheld fluorometer for accurate quantitative measurement.
- Precise and linear detection range: 1 – 100 ppb.



Assay Procedure:

Important: Prior to assay, bring the assay components to room temperature.
Overview: Total Aflatoxin Assay Kit uses a simple procedure to purify aflatoxins from food samples and rapidly determine their concentration using a fluorometer. A liquid extract prepared from the sample is passed through the column to bind the aflatoxins. After washing the column with a wash solution, the aflatoxins are eluted using methanol. The eluate is mixed with the Derivitizing Reagent to increase the aflatoxin fluorescence and measured on a fluorometer calibrated with the Total Aflatoxin Standard included in the kit. See attached sheet for detailed procedures for different food types.

1. Column preparation. (See next sheet.)
2. Extraction. (See next sheet.)
3. Chromatography. (See next sheet.)
4. Pre-clean mini-glass tubes: According to the usage and prepare enough mini-glass tubes. Add 200uL of methanol in each tube, let sit for 1 minute, and remove the solution. Repeat 2-3 times to make sure no residue is left in the tube.
5. According to the usage, dilute the 10x Derivitizing Reagent by 10 times. (e.g. 1-mL with 9-mL dH2O.) Use within 8 hours.
6. Sample tube preparation: Add 100 µL of final eluate into a mini-glass tube. Add 100 µL of diluted 1x Derivitizing Reagent and mix by pipetting 5-10 times.
7. Standard tube preparation: Pipette 100-150 µL of included 100-ppb Total Aflatoxin fluorescence equivalent solution into a mini-glass tube.
8. Blank tube preparation: Pipette 100 µL of methanol into a mini-glass tube and add 100 µL of diluted 1x Derivitizing Reagent and mix by pipetting 5-10 times.
9. Switch on the fluorometer for 5 minutes. To calibrate the reader, place the "Blank" tube into the sample chamber. Press [Calibrate]→[Confirm]→[Assay 1]→[Blank]. Reader starts Measuring. After blank is finished, press the number setting keys [< + - >] to change the value until the window shows "000100.00". Place the “Standard” tube into the sample chamber. Press "Measure Std". The reader shows "Calibrate Finished". The fluorometer is now calibrated. Press [Return].
10. Put the “Sample” tube in the sample chamber, then press [Measure] → [Assay 1] → [Measure]. The aflatoxin concentration will be displayed in the window. Multiply the dilution factor used for extracting and purifying the aflatoxins to obtain total aflatoxin concentration of your food sample. Record the data, or press [Save] to save the data for later retrieval. Press [Return] and then [Measure] for the next sample.

Note:

1. Use lint-free wipe to clean the outside wall of the mini-glass tubes before each measurement to ensure accuracy.
2. You can measure each sample 3-5 times and take the average to increase the accuracy. Wait for 10 seconds and rotate the mini-glass tube between each measurement to average out the system variation.
3. You can also make triplicate of sample tubes from the same final elute to average out the tube variation.
4. Use HPLC-grade methanol and distilled or de-ionized water to avoid potential fluorescence interference.
5. If “Sample” concentration is higher than the upper limit, dilute Sample in methanol and repeat measurement.

Product Information:

- Assay Kit: Sufficient for about 25 tests. Kit content: (1) columns (25ea), (2) 10X Derivitizing Reagent (3mL), (3) Total Aflatoxin 100-ppb fluorescence equivalent solution (5mL). (4) 100 mini glass tubes.
- Fluorometer. The reader comes with a 5VDC power adapter, a USB cable, manual and data management software CD.
- Avoid contact and inhalation. Standard laboratory safety procedures should be followed when handling this product. Safety procedures include wearing OSHA approved safety glasses, gloves and protective clothing.
- *Shipping and storage*: the kit is shipped on ice-pack. Store Assay at 4°C. Shelf life of 18 months after receipt. Don’t store columns above 30 °C.

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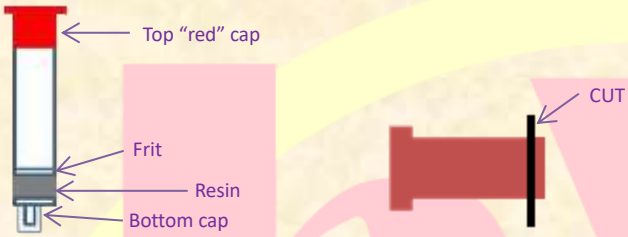
Column Preparation Procedures:

Diagram of Column



1. Remove a column from the box and bring to room temperature before running any samples. Pull out the red cap, and place column into a column rack.
2. With scissors or a razor blade, cut the closed end of the cap, removing the smallest amount of material possible from the closed bottom end of the cap, in order to make the cap an open tube.
3. Place the cap back onto the column.
4. This will allow you to form a good seal between the column and a 25 mL serological pipette. A good seal will aid in using a 25 mL serological pipette for applying positive pressure on the column if necessary to maintain proper flow rates.

Applying Positive Pressure:

Flow rates listed in the chromatography instructions should be followed carefully; this may require the use of positive pressure on the column to maintain the proper flow rate. This can be accomplished by using a manual serological pipette controller and a 25 mL serological pipette in the following manner:

1. Place 25 mL serological pipette into the manual pipette controller.
2. Draw air into the manual pipette controller until maximum volume is reached.
3. Place the serological pipette into the top red cap of the column firmly, until an airtight seal has been made.
4. Slowly expel air from the serological pipette into the column. This will create positive pressure increasing the flow rate of the column.

Note: In general, applying positive pressure will not be required if the extraction and chromatography procedure is followed correctly.

Derivatizing Reagent dilution:

- Mix 3mL of Derivatizing Reagent with 27mL distilled or deionized water. If kept away from direct sunlight the diluted reagent will last 8hours.
- Smaller volumes of Derivatizing Reagent can be mixed as needed at a 1:9 ratio (i.e. 1mL Derivatizing Reagent and 9 mL water).

100ppb Total Aflatoxin fluorescence equivalent solution:

- The Total Aflatoxin 100-ppb fluorescence equivalent solution is ready to use as supplied.

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Food Sample Extraction and Chromatography Procedures:

Nuts (peanuts, cashews, pecans, walnuts, almonds): (Dilution factor: 4)

1. Extraction

- Weigh 25g of ground or homogenized sample, add 5 g NaCl and place in a blender.
- Add 100 mL methanol-water (70%:30% v/v) mixture. Blend at high speed for 2 minutes.
- Pour about 20 mL of extract over a Whatman#40 filter paper in a glass funnel and collect filtrate in a clean tube.
- Transfer 5 mL of filtered extract into a clean 50-mL tube containing 10 mL of deionized water, and vortex for 1 minute at maximum speed.
- Pour this mixture over a glass microfiber filter paper in a glass funnel into a clean 15-mL tube.

2. Chromatography

- Refer to the column preparation procedure, then remove the bottom cap from the column. Discard the liquid from the top portion of the prepared column. Tap the bottom opening of the column 5 times on a dry paper towel to remove extra liquid.
- Pass a total of 6 mL filtered dilute extract completely through the column at a rate of no more than 1-2 drop per second. Add the sample as to not overfill the column in 2 x 3-mL portions.
- Pass 6 mL methanol-water (23%:67% v/v) mixture through the column at a rate of no more than 1-2 drops per second. Add the liquid as to not overfill the column in 2 x 3-mL portions. Tap the bottom opening of the column 10 times on a dry paper towel to expel all liquid inside.
- To elute the aflatoxin from the column, add 2 mL HPLC grade methanol into the top of the column at a flow rate of no more than 1 drop per second. This is your final elution for HPLC analyis.

Corn, peanut butter & peanut oil: (Dilution factor: 4)

1. Extraction

- Weigh 25g of ground or homogenized sample, add 5 g NaCl and place in a blender.
- Add 100 mL methanol-water (70%:30% v/v) mixture. Blend at high speed for 2 minutes.
- Pour about 20 mL of extract over a Whatman#40 filter paper in a glass funnel and collect filtrate in a clean tube.
- Transfer 5 mL of filtered extract into a clean 50-mL tube containing 10 mL of deionized water, and vortex for 1 minute at maximum speed.
- Pour this mixture over a glass microfiber filter paper in a glass funnel into a clean 15-mL tube.

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- Refer to the column preparation procedure, then remove the bottom cap from the column. Discard the liquid from the top portion of the prepared column. Tap the bottom opening of the column 5 times on a dry paper towel to remove extra liquid.
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