PerkinElmer®
For the Better

MaxSignal® IAC for Total Aflatoxin

Aflatoxin $B_1/B_2/G_1/G_2$ Immunoaffinity Column

Catalog #FOOD-1514-01

ISO 9001 QUALITY ASSURANCE

Manufactured in compliance with our ISO 9001 certified quality management system.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA

P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

RELATED PRODUCTS

CATALOG #	PRODUCT	QTY
FOOD-1000-00	AuroFlow™ X	00 strips
FOOD-1000-00	MaxSignal® X	00

GENERAL INFORMATION

Purpose

The immunoaffinity column can selectively adsorb aflatoxins (B_1, B_2, G_1, G_2) from the sample solution, thereby purifying the sample. The purified sample solution can then be directly used for HPLC analysis.

Affinity columns can be used in combination with HPLC to achieve rapid testing, and to increase signal-to-noise ratio and improve the accuracy of the detection method.

Overview

Aflatoxins are toxic metabolites of a class of fungi (such as *Aspergillus flavus* and *Aspergillus parasiticus*). They are highly carcinogenic and are found mainly in grains, peanuts, nuts, cottonseeds, animal feed, vegetable oils, as well as animal tissues and blood. Among them, Aflatoxin B_1 (AFT B_1) ranks first in terms of toxicity, carcinogenicity and frequency of contamination.

Principle

The basis of the measurement is the antigen-antibody reaction. Antibodies are bound in the column and the aflatoxin in the sample is extracted, filtered, and diluted, and then passed slowly through the aflatoxin immunoaffinity column. The toxins bind to the antibodies in the column and the immunoaffinity column is then washed to remove other unrelated substances that have not been bound. Aflatoxin is then eluted with methanol and injected into an analytical instrument for detection.

KIT CONTENTS, STORAGE, & SHELF LIFE

Each kit contains Aflatoxin immunoaffinity columns of various specifications and 1 instruction manual. Store the entire kit at 2–8°C. Do not use this product past the expiration date indicated on the Certificate of Analysis.

Required Materials Not Provided with the Kit

- HPLC
- Derivatization device: such as optical derivatization device, photochemical derivatization device, iodine derivatization device
- Centrifuge capable of at least 3,000-4,000 x g
- Nitrogen gas evaporator apparatus
- Nitrogen gas tank and pressure regulator
- LC-MS (LC-MS/MS)
- Air-pressure controller
- Air pump
- Balance with 0.01 g readability
- High-speed homogenizer (i.e. rotary shaker, vortexer, stomacher, or equivalent) (maximum speed ≥ 10,000 RPM)
- Grinder
- Sieving screen: 2-mm
- pH meter (or pH test paper)
- Graduated cylinder: 10 mL & 100 mL
- Funnel: 50 mL
- Syringe: 10 mL & 20 mL
- Pipette and pipette tips
- Homogenization flask (or 250-mL conical flask with pestle)
- Sample tubes and bottles
- Qualitative filter paper
- Microfiber filter paper (e.g. Whatman 934-AH)
- Column holder and syringe connector plug (for use with 6-mL immunoaffinity columns)
- Methanol (CH_3OH): Chromatography Grade
- Acetonitrile (CH_3CN): Analytical Grade
- Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$): Analytical Grade
- Acetic acid (CH_3COOH): Chromatography Grade
- Potassium dihydrogen phosphate (KH2PO4): Analytical Grade
- Potassium chloride (KCl): Analytical Grade
- Sodium chloride (NaCl): Analytical Grade
- Tween-20® ($C_{58}H_{114}O_{26}$): Analytical Grade
- Hydrochloric acid (HCl): Analytical Grade
- Sodium hydroxide (NaOH): Analytical Grade
- Distilled/deionized water

PRECAUTIONS

- Allow the immunoaffinity column to equilibrate to room temperature (20–25°C) before use.
- The immunoaffinity column should be stored at 2–8°C; do not freeze.
- Do not use any expired immunoaffinity column.
- The sample volume can be increased or decreased appropriately as needed. The volume of the extraction solution should be adjusted accordingly.
- The pH of the loading solution onto the immunoaffinity column should be 6–8. If it deviates from this range, the pH should be adjusted with dilute hydrochloric acid or dilute sodium hydroxide.
- Maintaining consistency (such as polarity, pH, and concentration) between the test solvent loaded into any analytical instrument and the mobile phase can help eliminate any adverse solvent effects.
- Column capacity: 300 ng, when the content of the toxin in the sample divided by the dilution factor is higher than the column capacity, it is necessary to reduce the volume of the sample solution appropriately, and retest.
- WARNING: Aflatoxin is toxic and carcinogenic; protective equipment such as gloves and masks should always be used during handling.
- Vessels and tools used to handle toxin solutions should be completely immersed in a sodium hypochlorite solution (5% v/v) overnight.
- Ensure the LC-MS/MS is clean and the tubing is primed appropriately for each run.
- Follow appropriate instrument precautions if using HPLC.

REAGENT PREPARATION

1. **Preparation of Extraction Solution 3: 70% v/v Methanol-water**
   Combine 700 mL of methanol and 300 mL of distilled/deionized water. Bring to 1 L final volume with distilled/deionized water. Mix well.
2. **Preparation of Extraction Solution: 80% v/v Acetonitrile-water**
   Combine 800 mL of acetonitrile and 200 mL of distilled/deionized water. Bring to 1 L final volume with distilled/deionized water. Mix well.
3. **Preparation of 1% Tween-water solution**
   Combine 1 mL of Tween-20, with distilled/deionized water to a final volume of 100 mL.

SAMPLE PREPARATION

Method 1 – Suitable for samples of grain crops such as corn, wheat, nuts, corn oil, flower oil, feed, etc.

1. Weigh out 25 g ± 0.01 g of sample in a bottle. Add 5 g of sodium chloride (NaCl) and 125 mL of Extraction Solution 3. Solid samples should be homogenized to pass through a 2-mm sieve before use.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes. Filter with microfiber filter paper. Collect the filtrate.
3. Combine 10 mL of the eluent with 20 mL of distilled/deionized water to dilute, then filter with microfiber filter paper, and collect the filtrate as sample solution. Use 15 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor = 1

Method 2 – Suitable for samples of flour, wheat flour, chili, pepper, soybean paste, sesame oil and other vegetable oils, straw, peanuts and their products

1. Weigh 25 g ± 0.01 g of sample into a bottle. Add 125 mL of Extraction Solution. Solid samples should be homogenized to pass through a 2-mm sieve.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes. Filter with microfiber filter paper. Collect the filtrate.
3. Combine 10 mL of the eluent with 70 mL of distilled/deionized water to dilute, then filter with microfiber filter paper, and collect the filtrate as sample solution. Use 20 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor = 2

Method 3 – Suitable for Chinese medicine (19 types of Chinese medicine specified in Version 2015 of the Pharmacopoeia)

1. Weigh out 15 g ± 0.01 g of sample (solid samples should be ground and passed through a 1-mm sieving screen) and add to 75 mL of Extraction Solution (80%acetonitrile-water solution).
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute or shake vigorously on a shaker (200-300 RPM) for 20 minutes. Filter with microfiber filter paper. Collect the filtrate.
3. Combine 10 mL of the filtrate with 40 mL of 1% Tween-water solution, then filter with microfiber filter paper, and collect the filtrate as sample solution.
4. Use 25 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor = 1

Method 4 – Suitable for tea samples such as black tea, green tea, oolong tea, and Pu'er tea

1. Weigh 5 g ± 0.01 g of sample into a bottle. Add 25 mL of Extraction Solution 1. Solid samples should be homogenized to pass through a 1-mm sieve before use.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes. Filter with microfiber filter paper. Collect the filtrate.
3. Combine 10 mL of the filtrate with 70 mL of distilled/deionized water. Mix well. Filter with microfiber filter paper, and collect the filtrate.
4. Use 20 mL of the filtrate as the final sample for testing.

Dilution factor = 2

OPERATING PROCEDURE

1. Remove the column and place into a column holder. Remove the plunger of a syringe, then attach the syringe through the connector plug above the column to complete the connection. Secure to an air-pressure controller, if available.
2. Transfer the appropriate amount of the solution processed in Sample Preparation to fill the syringe.
3. Remove the cap under the affinity column (do not discard as this will be used in the next step). Adjust the air-pressure to have a flow rate of 1–2 drops/second.
4. After all the liquid has flowed through, add 10 mL of water to wash the column at a flow rate of 2–3 drops per second. Repeat this wash step one more time. Note: if the column appears darker due to the material passed through, pre-wash one time with 10 mL of 1% Tween-water Solution before washing with water.
5. After the liquid has completely flowed through add 1 mL of methanol at a flow rate of 1 drop per second, and collect the eluate and bring to volume of 1 mL.
6. Filter the eluent through a 0.22 µm micropore filter and then transfer into a sample bottle to be used for HPLC analysis.

Also applicable to GB 5009.22-2016, GB/T30955-2014 and other pharmacopoeial methods.

INTERPRETATION OF RESULTS

Aflatoxin Concentration = Detected Concentration x Dilution Factor

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